PI: **RAFOLS, JOSE A**	Title: Control of microvascular tone in traumatic brain injury	
Received: 06/29/2009	FOA: PA07-070	Council: 01/2010
Competition ID: ADOBE-FORMS-A	FOA Title: RESEARCH PROJECT GRANT (PARENT R01)	
2 R01 NS039860-09A2	Dual:	Accession Number: 3207999
IPF: 9110501	Organization: WAYNE STATE UNIVERSITY	
Former Number:	Department: Anatomy and Cell Biology	
IRG/SRG: BINP	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 9: 250,000 Year 10: 250,000 Year 11: 250,000 Year 12: 250,000 Year 13: 250,000	Animals: Y Humans: N Clinical Trial: N Current HS Code: 10 HESC: N	New Investigator: N Early Stage Investigator: N
Senior/Key Personnel:	*Organization:*	*Role Category:*
Jose Rafols Ph.D.	Wayne State University	PD/PI
Christian Kreipke PhD	Wayne State University	Other (Specify)-CO-Investigator
Mihir Bagchi PhD	Wayne State University	Other (Specify)-Co-Investigator
Donald Kuhn PhD	Wayne State University	Consultant
Ewart Haacke PhD	Wayne State University	Consultant

Additions for Review

Accepted Publication New publication 08/31/2009

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

2. DATE SUBMITTED

Applicant Identifier

3. DATE RECEIVED BY STATE

State Application Identifier

1. * TYPE OF SUBMISSION

☐ Pre-application ☐ Application ☒ Changed/Corrected Application

4. Federal Identifier NS039860

5. APPLICANT INFORMATION

*** Organizational DUNS:** 001962224

* Legal Name: Wayne State University

Department: | Division:

* Street1: 5057 Woodward

Street2:

* City: Detroit | County:

* State: MI: Michigan | Province:

* Country: USA: UNITED STATES | * ZIP / Postal Code: 48202

Person to be contacted on matters involving this application

Prefix: Ms. | * First Name: Gail | Middle Name:

* Last Name: Ryan | Suffix:

* Phone Number: 313.577.2294 | Fax Number: 313.577.2653

Email: orspsmail@wayne.edu

6. * EMPLOYER IDENTIFICATION *(EIN) or (TIN):* 38-6028429

7. * TYPE OF APPLICANT: H: Public/State Controlled Institution of Higher Education

Other (Specify):

Small Business Organization Type ☐ Women Owned ☐ Socially and Economically Disadvantaged

8. * TYPE OF APPLICATION:

☐ New ☒ Resubmission

☐ Renewal ☐ Continuation ☐ Revision

If Revision, mark appropriate box(es).

☐ A. Increase Award ☐ B. Decrease Award ☐ C. Increase Duration ☐ D. Decrease Duration

☐ E. Other (specify):

* Is this application being submitted to other agencies? Yes ☐ No ☒ What other Agencies?

9. * NAME OF FEDERAL AGENCY:

National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER:

TITLE:

11. * DESCRIPTIVE TITLE OF APPLICANT'S PROJECT:

Control of microvascular tone in traumatic brain injury

12. * AREAS AFFECTED BY PROJECT *(cities, counties, states, etc.)*

Detroit, Wayne, Michigan

13. PROPOSED PROJECT:

* Start Date: 05/01/2010 | * Ending Date: 04/30/2015

14. CONGRESSIONAL DISTRICTS OF:

a. * Applicant: MI-013 | b. * Project: MI-013

15. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: Dr. | * First Name: Jose | Middle Name: A.

* Last Name: Rafols | Suffix: Ph.D.

Position/Title: Professor

* Organization Name: Wayne State University

Department: Anatomy and Cell Biology | Division: Medicine

* Street1: 540 E Canfield

Street2:

* City: Detroit | County: Wayne

* State: MI: Michigan | Province:

* Country: USA: UNITED STATES | * ZIP / Postal Code: 48201

* Phone Number: 313.993.4393 | Fax Number: 313.577.3125

* Email: jrafols@med.wayne.edu

OMB Number: 4040-0001
Expiration Date: 04/30/2008

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

16. ESTIMATED PROJECT FUNDING

a. * Total Estimated Project Funding: 1,900,000.00

b. * Total Federal & Non-Federal Funds: 1,900,000.00

c. * Estimated Program Income: 0.00

17. * IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?

a. YES ☐ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:

DATE:

b. NO ☒ PROGRAM IS NOT COVERED BY E.O. 12372; OR

☐ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

18. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

☒ *** I agree**

** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.*

19. Authorized Representative

Prefix: Ms. * First Name: April Middle Name:

* Last Name: Spraggins Suffix:

* Position/Title: Grant & Contract Officer III

* Organization: Wayne State University

Department: Sponsored Programs Administrat Division:

* Street1: 5057 Woodward

Street2: 13th Floor

* City: Detroit County: Wayne

* State: MI: Michigan Province:

* Country: USA: UNITED STATES * ZIP / Postal Code: 48202

* Phone Number: 313.577.1445 Fax Number: 313.577.1348

* Email: au0477@wayne.edu

*** Signature of Authorized Representative**: April Spraggins

*** Date Signed**: 06/29/2009

20. Pre-application Add Attachment Delete Attachment View Attachment

21. Attach an additional list of Project Congressional Districts if needed.

Add Attachment Delete Attachment View Attachment

OMB Number: 4040-0001
Expiration Date: 04/30/2008

424 R&R and PHS-398 Specific Table Of Contents

RESEARCH & RELATED Project/Performance Site Location(s)

Project/Performance Site Primary Location

Organization Name: Wayne State University

* Street1: 540 East Canfield

Street2: Rooms 9312, 9320, 9332 Scott Hall

* City: Detroit County: Wayne

* State: MI: Michigan Province:

* Country: USA: UNITED STATES * ZIP / Postal Code: 48201

Project/Performance Site Location 1

Organization Name:

* Street1:

Street2:

* City: County:

* State: Province:

* Country: USA: UNITED STATES * ZIP / Postal Code:

Additional Location(s) Add Attachment Delete Attachment View Attachment

OMB Number: 4040-0001
Expiration Date: 04/30/2008

Close Form | Print Page | About

RESEARCH & RELATED Other Project Information

1. * Are Human Subjects Involved? ☐ Yes ☒ No

1.a If YES to Human Subjects

Is the IRB review Pending? ☐ Yes ☐ No

IRB Approval Date:

Exemption Number: ☐1 ☐2 ☐3 ☐4 ☐5 ☐6

Human Subject Assurance Number:

2. * Are Vertebrate Animals Used? ☒ Yes ☐ No

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ☐ Yes ☒ No

IACUC Approval Date: 02/01/2008

Animal Welfare Assurance Number A3310-01

3. * Is proprietary/privileged information included in the application? ☐ Yes ☒ No

4.a. * Does this project have an actual or potential impact on the environment? ☐ Yes ☒ No

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ☐ Yes ☐ No

4.d. If yes, please explain:

5.a. * Does this project involve activities outside the U.S. or partnership with International Collaborators? ☐ Yes ☒ No

5.b. If yes, identify countries:

5.c. Optional Explanation:

6. * Project Summary/Abstract 1243-Abstract.pdf — Add Attachment | Delete Attachment | View Attachment

7. * Project Narrative 1247-Project Narrative.pdf — Add Attachment | Delete Attachment | View Attachment

8. Bibliography & References Cited 1246-References Cited R01.pdf — Add Attachment | Delete Attachment | View Attachment

9. Facilities & Other Resources 1244-Facilities and other Resources.p — Add Attachment | Delete Attachment | View Attachment

10. Equipment 1245-Equipment.pdf — Add Attachment | Delete Attachment | View Attachment

11. Other Attachments Add Attachments | Delete Attachments | View Attachments ☐

OMB Number: 4040-0001
Expiration Date: 04/30/2008

Abstract

A major event following traumatic brain injury (TBI) is the loss of autoregulatory capacity of brain microvessels which results in sustained hypoperfusion and improper delivery of vital metabolites to brain tissue. Previously we established that the sustained hypoperfusion and the lack of autoregulatory capacity post trauma are largely due to the capability of injured cells in the vascular wall and in other brain compartments to synthesize and maintain excessive amounts of endothelin-1 (ET-1). ET-1, as well as its two isoforms ET-2 and ET-3, exert signal-transducing, vasoconstrictor effects through their only known receptors ET-RA and ET-RB. Although activation of ET-RA is known to cause vasoconstriction and ET-RB causes vasodilation in both in vitro preparations and non-neural tissues, little is known of their role in the injured brain and the development of secondary cell injury. In the current funding period we established that ET-RA is upregulated at 4 hours, while ET-RB is upregulated 24 hours following TBI. Further, blockade of ET-RA activation either through inhibition of gene expression or pharmacological antagonism prior to TBI blocks hypoperfusion. Blockade of ET-RB, on the other hand, had no effect. While this data provides valuable information about the mechanisms underlying enhanced vascular tone leading to hypoperfusion following trauma, the current proposal seeks to establish the effectiveness of ET-RA or ET-RB antagonism in both ameliorating hypoperfusion and improving behavioral outcome. Specifically, ET-RA and ET-RB antagonists, including an ET-RA antagonist currently undergoing clinical trial, will be delivered via intracerebroventricular injections directly following TBI. CBF, microvascular luminal area, ET-RA and B expression, and cognitive outcome will be measured to determine the effects of ET-RA and B blockade following TBI. Collectively, this data will be analyzed to determine whether blockade of endothelin receptors may be effective in the development of therapeutic strategies used to treat those suffering from the effects of TBI.

Project Narrative

Traumatic brain injury (TBI) is the leading cause of death and disability amongst our youth and children. Further, it has been named as the signature injury in the War on Terrorism that, upon return of our men and women fighting in Iraq and Afghanistan, is projected to cost millions in patient care and rehabilitation costs. While TBI results in three major pathologies, including diffuse axonal injury, brain edema, and hypoperfusion of the brain's parenchyma, this proposal investigates novel methods to increase blood flow after injury. In doing so, the experiments in this proposal are designed to yield results that can quickly be translated into the clinical setting, thus off-setting the current potentially dismal outcome following exposure to TBI.

Facilities and other Resources

The laboratory consists of approximately 700 sq.ft. in which animal perfusion, tissue processing, sectioning and analysis can be performed. There is also an equipment room where the surgeries are conducted and which houses the trauma model. Drs. Rafols and Kreipke have an additional laboratory of 700 sq.ft. that is used for behavioral testing.

The office facilities are located adjacent to the laboratory. The Principal Investigator and both Co-investigators have their own office space fully equipped with computers and computer peripheria.

Facilities for the care and housing of experimental animals are available in the basement of Scott Hall (the same building as the other laboratories). These resources are operated by the University Department of Laboratory Animal Resources. At WSU, all animals used for biomedical research at the medical center are housed in modern animal care facilities with excellent supervisory and veterinary support.

Equipment:

Three cryostats are available in the Department of Anatomy and Cell Biology; balance, ph meter, freezer, 2 refrigerators, dessicator, and equipment for ICC and Western blots are also available. All behavioral equipment including raidal arm maze, treadmills, motor testing equipment are also currently available. Dr. Haacke, as indicated in his letter of support, will provide full access to MRI facilities.

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix:	Dr.
* First Name:	Jose
Middle Name:	A.
* Last Name:	Rafols
Suffix:	Ph.D.
Position/Title:	Professor
Department:	Anatomy and Cell Biology
Organization Name:	Wayne State University
Division:	Medicine
* Street1:	540 E Canfield
Street2:	
* City:	Detroit
County:	Wayne
* State:	MI: Michigan
Province:	
* Country:	USA: UNITED STATES
* Zip / Postal Code:	48201
* Phone Number:	313.993.4393
Fax Number:	313.577.3125
* E-Mail:	jrafols@med.wayne.edu
Credential, e.g., agency login:	JOSERAFOLS
* Project Role:	PD/PI
Other Project Role Category:	
*Attach Biographical Sketch	1234-X-biosketch-Rafols.pdf
Attach Current & Pending Support	1235-Ongoing Research Support

Add Attachment Delete Attachment View Attachment
Add Attachment Delete Attachment View Attachment

PROFILE - Senior/Key Person 1

Prefix:	Dr.
* First Name:	Christian
Middle Name:	William
* Last Name:	Kreipke
Suffix:	PhD
Position/Title:	Assistant Professor
Department:	Anatomy and Cell Biology
Organization Name:	Wayne State University
Division:	Medicine
* Street1:	540 E Canfield
Street2:	
* City:	Detroit
County:	Wayne
* State:	MI: Michigan
Province:	
* Country:	USA: UNITED STATES
* Zip / Postal Code:	48201
* Phone Number:	313.577.1049
Fax Number:	313.577.3125
* E-Mail:	ckreipke@med.wayne.edu
Credential, e.g., agency login:	aa5930
* Project Role:	Other (Specify)
Other Project Role Category:	CO-Investigator
*Attach Biographical Sketch	1236-X-biosketch-Kreipke.pdf
Attach Current & Pending Support	1237-Ongoing Research Support

Add Attachment Delete Attachment View Attachment
Add Attachment Delete Attachment View Attachment

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Senior/Key Person 2

Prefix: Dr.	* First Name: Mihir	Middle Name:	
* Last Name: Bagchi		Suffix: PhD	
Position/Title: Associate Professor		Department: Anatomy and Cell Biology	
Organization Name: Wayne State University		Division: Medicine	
* Street1: 540 East Canfield			
Street2:			
* City: Detroit	County: Wayne		
* State: MI: Michigan		Province:	
* Country: USA: UNITED STATES		* Zip / Postal Code: 48201	
* Phone Number: 313.577.0574	Fax Number: 313.577.3125		
* E-Mail: mbagchi@med.wayne.edu			
Credential, e.g., agency login:			
*** Project Role:** Other (Specify)	**Other Project Role Category:** Co-Investigator		
***Attach Biographical Sketch**	1238-X_biosketch_Bagchi.pdf	Add Attachment / Delete Attachment / View Attachment	
Attach Current & Pending Support		Add Attachment / Delete Attachment / View Attachment	

PROFILE - Senior/Key Person 3

Prefix: Dr.	* First Name: Donald	Middle Name:	
* Last Name: Kuhn		Suffix: PhD	
Position/Title: Professor		Department: Psychiatry	
Organization Name: Wayne State University		Division: Medicine	
* Street1: 540 East Canfield			
Street2:			
* City: Detroit	County: Wayne		
* State: MI: Michigan		Province:	
* Country: USA: UNITED STATES		* Zip / Postal Code: 48201	
* Phone Number: 313.576.4525	Fax Number:		
* E-Mail: donald.kuhn@wayne.edu			
Credential, e.g., agency login: aa3071			
*** Project Role:** Consultant	**Other Project Role Category:**		
***Attach Biographical Sketch**	1239-X-biosketch-Kuhn.pdf	Add Attachment / Delete Attachment / View Attachment	
Attach Current & Pending Support	1240-Ongoing Research Support	Add Attachment / Delete Attachment / View Attachment	

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Senior/Key Person 4

Prefix:	Dr.
* First Name:	Ewart
Middle Name:	Mark
* Last Name:	Haacke
Suffix:	PhD
Position/Title:	Professor
Department:	Radiology
Organization Name:	Wayne State University
Division:	Medicine
* Street1:	540 East Canfield
Street2:	
* City:	Detroit
County:	Wayne
* State:	MI: Michigan
Province:	
* Country:	USA: UNITED STATES
* Zip / Postal Code:	48201
* Phone Number:	313.577.0574
Fax Number:	
* E-Mail:	nmrimaging@aol.com
Credential, e.g., agency login:	ak5444
*** Project Role:**	Consultant
Other Project Role Category:	

***Attach Biographical Sketch**	1241-X-biosketch-Haacke.pdf	Add Attachment	Delete Attachment	View Attachment
Attach Current & Pending Support	1242-Ongoing Research Support	Add Attachment	Delete Attachment	View Attachment

ADDITIONAL SENIOR/KEY PERSON PROFILE(S)		Add Attachment	Delete Attachment	View Attachment
Additional Biographical Sketch(es) (Senior/Key Person)		Add Attachment	Delete Attachment	View Attachment
Additional Current and Pending Support(s)		Add Attachment	Delete Attachment	View Attachment

OMB Number: 4040-0001
Expiration Date: 04/30/2008

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME Jose A. Rafols	POSITION TITLE Professor
eRA COMMONS USER NAME	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Illinois Benedictine, Lisle, IL	B.S.	1965	Biology
University of Kansas, Kansas City, KS	Ph.D.	1969	Anatomy
S. Ramon Y Cajal Institute, CSIC, Madrid, Spain	Post Doc	1970	Neuroanatomy

A. Positions and Honors

Positions and Employment

1969-1970 Instructor, Dept. of Anatomy/Cell Biology, Wayne State University, School of Medicine
1970 NIH Postdoctoral trainee at S. Ramon Y Cajal Institute, CSIC, Madrid, Spain
1971-1973 Asst. Professor, Dept. of Anatomy/Cell Biology, Wayne State University, School of Medicine
1973-1989 Assoc. Professor, Dept. of Anatomy/Cell Biology, Wayne State University, School of Medicine
1989-present Professor, Dept. of Anatomy/Cell Biology, Wayne State University, School of Medicine
1994-present Dir., Morphology and Imaging Core, Neurotrauma Center, Wayne State University, School of Medicine

Honors

DHHS/PHS/NIH Study Section Member (full member), Neurological Disorder Program Project Review A Committee (NSP-term) 7/1/90-6/30/94.
Chairman, Site visit, The Johns Hopkins University, Baltimore, MD; "Disorders of aging neuro-transmitter systems and neurotrophins", December 15-17, 1991.
Member, National Institutes of Health Reviewers Reserve (NRR), for term 7/1/94-6/30/98.
Member, American Heart Association National Study Committee, Brain Review Committee, for term 7/96-6/99.

B. Selected peer-reviewed publications (past five years)

Petrov T, Page AB, Owen C, Rafols JA. 2000 Expression of the inducible nitric oxide synthase in distince cellular types after traumatic brain injury. Acta Neuropathol 100:196-204.
Dore-Duffy P, Owen C, Bahabanov R, Murphy S, Rafols JA. 2000 Pericyte response to traumatic brain injury (TBI): Elongation and migration from the microvascular wall. Microvascular Res 60:55-69.
White BC, Sullivan JM, DeGracia DJ, O'Neill BJ, Neumar RW, Grossman LI, Rafols JA, Krause GS. 2000 Brain ischemia and reperfusion: Molecular mechanisms of neuronal injury. J Neurolog Sci 179:1-33.
Owen CR, Lipinski C, Page AB, White BC, Sullivan JM, DeGracia DJ, Rafols JA, Krause GS. 2001 Characterization of the eIF2ά-assocated protein p67 during brain ischemia and reperfusion. In Maturation Phenomen Cerebral Ischemia IV. Springer-Verlag, Berlin-Heidelberg pp. 19-24.
Petrov T, Underwood W, Alousi S, Own C, Rafols JA. 2001 Upregulation of iNOS and eIF2άP expression is paralleled by suppression of protein synthesis in the hypothalamus following trauma to the brain. J Neurotrauma 18:799-812.
Petrov T and Rafols JA. 2001 Acute alterations of endothelin-1 and iNOS expression and control of the brain microcirculation after head trauma. Neurol Res 23:138-143.
Balabanov R, Goldman R, Murphy S, Pellizon G, Own C, Rafols JA, Dore-Duffy P. 2001 Endothelial cell activation following moderate trauamatic brain injury. Neurol Res 23:175-182.
Barnes M, Lapanosky K, Rafols JA, Lawson DM, Dunbar JC. 2001 GnRH release is decreased in the absence of nitric oxide. PSEBM 226:701-706.

Petrov T, Steiner J, Braun B, Rafols JA. 2002 Sources of endothelin-1 in hippocampus and cortex following traumatic brain injury. Neurosci 115:275-283.

Barnes MJ, Lapanowski K, Rafols JA, Lawson DM, Dunbar JC. 2002 Chronic nitric oxide deficiency is associated with altered leutinizing hormone and follicle-stimulating hormone release in ovariectomized rats. PSEBM 233:817-822.

Ding Y, Li J, Phillis JW, Rafols JA, Diaz FG. 2002 Preperfusion infusion into ischemic territory reduces inflammatory injury after transient middle cerebral artery occlusion in rat. Stroke 33:2492-2498.

Ding Y, Li J, Lai Q, Azam S, Rafols JA, Diaz FG. 2002 Functional improvement after motor training is correlated with synaptic plasticity in rat thalamus. Neurol Res 24:829-836.

Petrov T, Rafols JA, Alousi SS, Kupsky WJ, Johnson R, Shah J, Shah A, Watson C. 2003 Cellular compartmentalization of Phosphorylated eIF2 alpha and neuronal NOS in human temporal lobe Epilepsy with hippocampal sclerosis. J Neurol Sci 209:31-39.

Ding Y, Li J, Clark J, Diaz FG, Rafols JA. 2003 Synaptic plasticity in thalamic nuclei enhanced by motor skill training in rat with transient middle cerebral artery occlusion. Neurol Res 25:189-194.

Ding Y, Li J, Rafols JA, Clark J, Phillis JW, Diaz FG. 2003 Preischemic motor exercise reduces ischemia/reprofusion injury in rats that correlates with regional angiogenesis and cellular expression of neurotrophin. Stroke 34:240-241.

Barnes MJ, Lapanowski K, Conley A, Rafols JA, Catherine KL, Dunbar JC. 2003 High fat feeding is associated with increased blood pressure, sympathetic nerve activity and hypothalamic mu opiod receptrs. Brain Res Bull 61:511-519.

Britton M, Rafols JA, Alousi S, Dunbar JC. 2003 The effects of middle cerebral artery occlusion on central nervous system apoptotic events in normal and diabetic rats. Experimental Diab Res 4:13-20.

Ding Y, Li J, Lai Q, Rafols JA, Luan X, Clark J, Diaz F. 2004 Motor balance and coordination training enhances functional outcome in rat with transient middle cerebral artery occlusion. Neuroscience 123:667-674.

Steiner J, Rafols D, Park H, Katar MS, Rafols JA, Petrov T. 2004 Attenuation of inOS mRNA exacerbates hypoperfusion and upregulates endothelin-1 expression in hippocampus and cortex after brain trauma. Nitric Oxide 10:162-169.

Rafols D, Steiner J, Rafols JA, Petrov T. 2004 Coexpression of iNOS and endothelin-1 mRNAs in specific cell types following traumatic brain injury. Neurosci letters 362:154-157.

Li J, Luan X, Clark J, Rafols JA, Diaz FG, Ding Y. 2004 Regional brain cooling induced by local saline infusion into ischemic territory produced a long-term neuroprotection in ischemic rats using a behavioral assessment. Neurol Res 26:677-683.

Ding Y, Li J, Rafols JA, Clark JC, McAllister JPII, Diaz FG, Guthikonda M, Ding Y. 2004 Exercise-induced angiogenic factors and reduction in ischemia/reperfusion injury. Current Neurovasc Res 1:411-420.

Dunbar J, Lapanowski K, Barnes M, Rafols JA. 2005 Hypothalamic agouti-related protein immunoreactivity in food-restricted, obese, and insulin-treated animals: evidence for glia cell localization. Exptl Neur 191:184-192.

Ding Y, Ding Y, Yojng C, Luan X, Li J, Rafols JA, Phillis JW, Calrck JC. 2005 Exercise pre-conditioning reduces inflammatory injury in ischemic rats during reperfusion. Acta neuropathol 109:237-246.

Kayali F, Montie H, Rafols JA, DeGracia DJ. 2005 Prolonged translation arrest in reperfused hippocampal Ca1 is mediated by Stress granules. Neurosci 134:1223-1245.

Kreipke C, Rafols J, Petrov T. 2005. Transcriptional and translational mechanisms for the reciprocal control of iNOS and endothelin 1 expression in brain microvessels after traumatic brain injury (TBI). Journal of Cerebral Blood Flow and Metabolism 25, S191.

Kreipke CW, Morgan N, Petrov T, Rafols J. 2006. Calponin and caldesmon cellular domains in reacting microvessels following traumatic brain injury. Microvascular Research. 71:197-204.

Kreipke CW, Morgan R, Petrov T, Rafols JA. 2007. Subcellular Redistribution of Calponin Underlies Sustained Vascular Contractility Following Traumatic Brain Injury. Neurol Res. 29:604-609.

Rafols J., Kreipke C, Petrov T. 2007. Alterations in Cerebral Cortex Microvessels and the Microcirculation in a Rat Model of Traumatic Brain Injury: a Correlative EM and Laser Doppler Flowmetry Study. Neurol Res 29:339-347.

Rafols J, Morgan R, Kallikuri S, Kreipke C. 2007. Extent of nerve cell injury in Marmarou's model compared to other brain trauma models. Neurol Res 29:348-355.

Degracia D, Kreipke C, Kayali F, Rafols JA. 2007. Brain endothelial HSP-70 stress response coincides with endothelial and pericyte death after brain trauma. Neurol Res 29:356-361.

Kallukuri S, Kreipke C, Rossi NF., Rafols JA, Petrov T. 2007. Spatial alterations in endothelin receptor expression are temporally associated with the altered microcirculation after brain trauma Endothelin receptor localization following traumatic brain injury. Neurol Res 29:362-368.

Kreipke C, Morgan R, Roberts G, Bagchi M, Rafols JA. 2007. Calponin phosphorylation in cerebral cortex microvessels mediates sustained vasoconstriction after brain trauma. Neurol Res 29:369-374.

Morgan R, Kreipke C, Robert G, Bagchi M, Rafols J. 2007. Neovascularization following traumatic brain injury: possible evidence for both angiogenesis and vasculogenesis. Neurol Res 29:375-381.

Kreipke CW, Morgan R, Kallakuri S, Rafols JA. 2007. Behavioral pre-conditioning enhances angiogenesis and cognitive outcome after brain trauma. Neurol Res. 29:388-94.

Dore-Duffy P, Kreipke C, Rafols JA. 2007. Differential expression of capillary VEGF isoforms following traumatic brain injury. Neurol Res 29:395-403.

Earlier Pertinent Publications

Rafols JA, Getchell TV. 1983 Morphological relations between the receptor neurons, sustentacular cells and Schwann cells in the olfactory mucosa of the salamander. Anat Rec 206:87-101.

Goshgarian HG, Rafols JA. 1984 The ultrastructural and synaptic architecture of phrenic motor neurons in the spinal cord of the adult rat. J Neurocytol 13:85-109.

Getchell ML, Rafols JA, Getchell TV. 1984 Histological and histochemical studies of the secretory components of the salamander olfactory mucosa: Effects of isoproterenol and olfactory nerve section. Anat Rec 208:553-565.

Rafols JA, Goshgarian H. 1985 Spinal tanycytes in the adult rat: A correlative Golgi—gold toning study. Anat Rec 211:75-86.

Rafols JA, Aronin N, DiFiglia M. 1986 A Golgi study of the monkey paraventricular nucleus: Neuronal types, afferent and efferent fibers. J comp Neur 257:585-613.

Rafols JA. 1986 Ependymal tanycytes of the ventricular system in vertebrates. In: Astrocytes: Development, Morphology and Regional Specialization of Astrocytes, vol. 1. Cellular Neurobiology: A series. S. Federoff and A. Vernadakis, Academic Press, Inc. Orlando, pp. 131-148.

Rafols JA and McNeill TH. 1987 Age-related dendritic changes of spiny and aspiny neurons in the rodent striatum. In the Basal Ganglia II: Structure and Funciton-Current Concepts. MB Carpenter and A JAyaraman (ed.s) Adv Behav Biol, vol 32, Plenum, New York, pp. 227-239.

McNeill TH, Brown SA, Rafols JA, Shoulson I. 1988 Regression of striatal dendrites in Parkinson's Disease. Brain Res 455:148-152.

Rafols JA, Cheng HW, McNeill TH. 1989 Golgi study of the mouse striatum: Age-related dendritic changes in different neuronal populations. J Comp Neur 279:212-227.

Goshgarian HC, YU XJ, Rafols JA. 1989 Neuronal and glial changes in the rat phrenic nucleus occurring within hours after spinal cord injury. J Comp Neur 284:519-533.

Ma TP, Cheng HW, Czech JA, Rafols JA. 1990 The intermediate and deep layers of the macaque superior colliculus: A Golgi study. J comp Neur 294:2-20.

McNeill TH, Koek LL, Brown SA, Rafols JA. 1991 Quantitative analysis of age-related dendritic changes in medium spiny I (MSI) striatal neurons of C57BL/6N mice. Neurobiol Aging 11:21-31.

Ma TP, Hu J, Anavi Y, Rafols JA. 1992 organization of the zona incerta in the macaque: Nissl and Golgi study. J Comp Neur 320:273-290.

White B, Daya A, DeGracia DJ, Krause G, Rafols JA. 1993 Fluorescent histochemical localization of lipid peroxidation during brain reperfusion following cardiac arrest. Acta Neuropath (Berlin) 86:1-90.

Crossland W, Hu X-J, Rafols JA. 1994 A morphological study of the rostral interstial nucleus of the medial longitude fasciculus in the monkey, *macaca mulatta*, by Nissl, Golgi, and Computer reconstruction methods. J comp Neurol 1:1-17.

Rafols JA, Daya AM, Krause GS, Neumar RW, White BC. 1995 Global brain ischemia and reperfusion: Golgi apparatus untrastructure in neurons selectively vulnerable ot death. Acta Neuropathol 90:17-30.

Rafols JA, Own C, Murphy S, Dore-Duffy P. 1995 Pericyte response following traumatic brain injury: Migration pericytes from CNS microvessels and apoptosis. J Neurotrauma 12:988 (vol 5).

O'Neil BJ, Krause LI, Grossman LI, Grunberger G, Rafols JA, DeGracia DJ, Newar BR, Tiffany BR, White BC. 1995 Global ischemia and reperfusion by cardiac arrest and resuscitation: Mechanisms leading to death of vulnerable neurons and a fundamental basis for therapeutic approaches. Cardiac Arrest: The Science of Practice of Resuscitation Medicine. Paradis, Halpern, and Nowak (eds.) Williams and Wilkins, Ch.5, pp. 84.

Ma TP, Lynch JC, Donahoe DK, Attallah H, Rafols JA. 1996 Organization of the medial pulvinar nucleus in the macaque. J Comp Neurol Anat Rec 250:220-237.

Page AB, Krause GS, Rafols JA. 1996 Differential expression of iNOS in rat cortex following trauma. Soc Neurosci (Abstracts) 22:2157.

Lenzi T, Raols JA. 1996 Reperfusion-induced changes in a nine-vessel occlusion model of ischemia. Soc NEurosci (Abstracts) 22:2157.

Neumar RW, Alousi SS, White BC, Rafols JA. 1996 Immunogold labeling of CaMKII in hippocampal neurons during global ischemia. Soc NEurosci (Abstracts) 22:1896.

Folkerts MM, Berman Wang G, Murphy S, Rafols JA, Muizelaar JP. 1996 Behavior morphological and electrophysiological effects of diffuse axonal injury in rats. J Neurotrauma 13:610.

Folkerts MM, Berman RF, Muizelaar JP, Rafols JA. 1998 Disruption of MAP2 immunostaining in rat hippocampus traumatic brain injury. J Neurotrauma 15:349-363.

Sullivan JM, Alousi SS, Kikade KR, Rafols JA, Krause GS, White BC. 1998 Insulin induces dephosphorylation of eIF2alpha(P) and restores protein synthesis in vulnerable hippocampal neurons following transient ischemia. J Cereb Blood Flow Metab 19:1010-1019.

O'Neil BJ, McKeown TR, DeGracia DJ, Alousi SS, Rafols JA, White BC. 1998 Cell death, calcium mobilization and immunostaining for phosphorylation eukaryotic initiation factor 2alpha in nueronally-differentiated NB-cells: Arachidonate and radical-mediated injury mechanisms. Resuscitation 41:71-83.

Goldstein EN, Own CR, White BC, Rafols JA. 1999 Ultrastructural localization of phosphorylated eIF2(alpha)P during brain reperfusion. Acta Neuropathol 98:493-505.

McKeown TR, Goldstein EN, Sullivan JM, Rafols JA, White BC, Krause GS. 1999 Nuclear localization and DNA binding properties of phosphorylated eIF2alpha (P) ub vulnerable hippocampal neurons during reperfusion. J Cereb Flow Metab 19:S510.

Rafols JA, Alousi SS, Owen CR, White BC, Sullivan JM. 1999 High doses of insulin do not prevent dephosphorylation of eIF2alpha(P), recovery of protein synthesis, and atrophy of hippocampal cA3 neurons during reperfusion. J Cereb Flow Metab 19:s511.

Petrov T, Own CR, Rafols JA. 1999 Differential synthesis of endothelin (ET-1) and nitric oxide (NO) in rat cereb microvessels following traumatic brain injury (TBI). Soc. Neurosci (Abstracts) 25:822.

Underwood BD, Lipinski CA, Rafols JA, Crossland WC, McAllister JP, Diaz FG, White BC. 1999 Effects of traumatic brain injury on phosphorylated eIF2alpha in the rat. Soc. Neurosci (abrstract) 25:820.

C. Ongoing Research Support

VA RR & D Award. Rossi (PI) 1/01/08-12/31/11
VA Rehabilitation
Role: CO-I (20% Effort)
"Conditioning, microvascular tone & rehabilitation post brain trauma"
Investigates the role of exercise in the control of microcirculation in a rat model of traumatic brain injury.

NIH-NINDS RO1 NS39860 06/01/04-05/31/09
Control of Microvascular tone in Traumatic Brain Injury.
The long term objective of this project is to investigate the effects of traumatic brain injury on the gene regulation and synthesis of molecules which effect contractility or relaxation of the smooth muscle cells in the wall of cerebral microvessels. The hypothesis being tested is that altered regulation of the genes that encode for endothelin receptors in endothelial cells, at different time points participate in the abnormal contractility of brain microvessels following trauma.
PI

NIH-NINDS RO1 NS044100 07/01/03-06/30/08
The Unfolded Protein Response after Brain Ischemia.
The proposal aims at investigating the unfolded-protein response as a mechanism of cell death in hippocampal CA1 neurons after brain ischemia/reperfusion.
Co-I

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME Christian W. Kreipke	POSITION TITLE Research Associate
eRA COMMONS USER NAME	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Wayne State University	B.A.	1995-1999	Anthropology
Wayne State University	M.A.	1999-2000	Medical Anthropology
Wayne State University, School of Medicine	Ph.D.	2000-2004	Neuroscience

A. Positions and Honors

01/97-05/97 Wayne State University, School of Medicine and Hutzel Hospital, Research Assistant, Bone Densitometry/Osteoporosis Project

09/97-09/99 Wayne State University, Institute for Information and Technology, Research Assistant, HIV/AIDS in Detroit Project

09/99-05/00 Wayne State University, Graduate Teaching Assistant, Department of Anthropology

05/00-09/00 Wayne State University, Adjunct Instructor, Department of Anthropology

09/00-08/04 Wayne State University, School of Medicine, Pre-Doctoral Research Assistant, National Institute of Drug Abuse T32 Training Grant

08/04-08/07 Wayne State University, School of Medicine, Research Associate, Dept. Anatomy and Cell Biology, Traumatic Brain Injury

08/07-present Wayne State University, School of Medicine, Assistant Professor (research), Dept. Anatomy and Cell Biology

Other Experience and Professional Memberships

05/99-present Member, Phi Beta Kappa

02/00-present Member, Society for Applied Anthropology

02/00-present Member, Society for Medical Anthropology

05/01-present Member, Sigma Xi

05/01-present Member, New York Academy of Sciences

03/01-03/02 Society for Neuroscience Brain Awareness Week Committee, Wayne State University, Chair

05/02-present Member, Society for Neuroscience

05/02-05/04 Michigan Society for Neuroscience, Student Counselor

05/03 Michigan Society for Neuroscience Chapter Meeting coordinator

11/04-08/07 Sigma Xi, Wayne State Chapter, Executive Board Member

02/05-08/07 Wayne State Alumni Communications Committee, Committee Member

05/06-08/07 Sigma Xi, National, Associate Director, NorthCentral Region

03/07-present Member, International Society for Cerebral Blood Flow and Metabolism

02/07-present Chairman of the Board, Southfield Oncology Institute

08/07-present Sigma Xi, National, Acting Director, NorthCentral Region

Honors

2002 Dean Thomas Asselin, M.D. Endowed Prize for Excellence in Psychiatry and Behavioral Neuroscience Research (Wayne State University School of Medicine)

2003 1st Place, Society for Neuroscience, MI Chapter, Poster Award

2006 Service Award For 2006 Sigma Xi National Conference

2007 Travel Award, Brain '07, Society for Cerebral Blood Flow and Metabolism

2007 Young Investigators Award, Endothelin 10, Endothelin

B. Peer-reviewed publications (in chronological order)

1. Kuhn DM, Sadidi M, Lu X, **Kreipke C**, Geddes T, Borges C, and Throck J. 2002 Peroxynitrite-Induced Nitration of Tyrosine Hydroxylase: Identification of Tyrosines 423, 428, and 432 as Sites of Modification by MALDI-TOF Mass Spectrometry and Tyrosine-Scanning Mutagenesis. Journal of Biological Chemistry 277:14336-14342.

2. **Kreipke C**, Walker PD. 2004. NMDA receptor blockade attenuates locomotion elicited by intrastriatal dopamine D1-receptor stimulation. Synapse 53:25-32.

3. **Kreipke C**, Rosenberg D, Keshavan M. 2004. Does disordered brain development cut across diagnostic boundaries? In Keshavan M, Kennedy J, Murray R (Eds.) Neurodevelopment and Schizophrenia. Cambridge University Press.

4. **Kreipke C**, Rafols J, Petrov T. 2005. Transcriptional and translational mechanisms for the reciprocal control of iNOS and endothelin 1 expression in brain microvessels after traumatic brain injury (TBI). Journal of Cerebral Blood Flow and Metabolism 25, S191.

5. **Kreipke CW**, Campbell BM, Walker PD. 2005. Failure of MK-801 to suppress D1 receptor-mediated induction of locomotor activity and striatal preprotachykinin mRNA expression in the dopamine-depleted rat. Neuroscience 137:505-517.

6. **Kreipke CW**, Morgan N, Petrov T, Rafols J. 2006. Calponin and caldesmon cellular domains in reacting microvessels following traumatic brain injury. Microvas Res. 71:197-204.

7. Shen Y, Kou Z, **Kreipke CW**, Petrov T, Hu J, Haacke EM. 2006. In vivo measurement of tissue damage, oxygen saturation changes and blood flow changes after experimental traumatic brain injury in rats using susceptibility-weighted imaging. Magn Reson Imaging 25(2):219-227.

8. **Kreipke CW**, Morgan R, Petrov T, Rafols JA. 2007. Subcellular Redistribution of Calponin Underlies Sustained Vascular Contractility Following Traumatic Brain Injury. Neurol Res. 29:604-609.

9. Petrov T, **Kreipke C**, Alilain W, Nantwi K. 2007. Differential Expression Adenosine A1 and A2 Receptor Protein Levels Following Upper Cervical (C2) Spinal Cord Hemisection In Adult Rats. J Spinal Cord Med 30:331-337.

10. Rafols J., **Kreipke C**, Petrov T. 2007. Alterations in Cerebral Cortex Microvessels and the Microcirculation in a Rat Model of Traumatic Brain Injury: a Correlative EM and Laser Doppler Flowmetry Study. Neurol Res 29:339-347.

11. Rafols J, Morgan R, Kallikuri S, **Kreipke C**. 2007. Extent of nerve cell injury in Marmarou's model compared to other brain trauma models. Neurol Res 29:348-355.

12. Degracia D, **Kreipke C,** Kayali F, Rafols JA. 2007. Brain endothelial HSP-70 stress response coincides with endothelial and pericyte death after brain trauma. Neurol Res 29:356-361.

13. Kallukuri S, **Kreipke C**, Rossi NF., Rafols JA, Petrov T. 2007. Spatial alterations in endothelin receptor expression are temporally associated with the altered microcirculation after brain trauma Endothelin receptor localization following traumatic brain injury. Neurol Res 29:362-368.

14. **Kreipke C**, Morgan R, Roberts G, Bagchi M, Rafols JA. 2007. Calponin phosphorylation in cerebral cortex microvessels mediates sustained vasoconstriction after brain trauma. Neurol Res 29:369-374.

15. Morgan R, **Kreipke C**, Robert G, Bagchi M, Rafols J. 2007. Neovascularization following traumatic brain injury: possible evidence for both angiogenesis and vasculogenesis. Neurol Res 29:375-381.

16. Kreipke CW, Morgan R, Kallakuri S, Rafols JA. 2007. Behavioral pre-conditioning enhances angiogenesis and cognitive outcome after brain trauma. Neurol Res. 29:388-94.

17. Dore-Duffy P, **Kreipke C**, Rafols JA. 2007. Differential expression of capillary VEGF isoforms following traumatic brain injury. Neurol Res 29:395-403.

18. Petrov T, Kreipke C, Alilain W, Nantwi KD. 2007. Differential expression of adenosine A1 and A2A receptors after upper cervical (C2) spinal cord hemisection in adult rats. J Spinal Cord Med. 30:331-337.

19. Huttemann M, Lee I, **Kreipke CW**, Petrov T. (in press). Suppression of iNOS prior to traumatic brain injury improves cytochrome oxidase activity and normalizes cellular energy levels. Neuroscience

20. Hoffman WH, Stamatovic SM, Rafols JA, **Kreipke CW**, Andjelkovic AV. (in press). Inflammatory mediators and blood brain barrier disruption in fatal brain edema of diabetic ketoacidosis. J Childhood Medicine.

21. **Kreipke CW**, Petrov T, Rafols JA. (in press). Endothelin A receptor antagonism blocks calponin phosphorylation following brain trauma. J Cereb Blood Flow and Metab.

22. Morgan R, **Kreipke CW**, Rafols JA. (in press). VEGFR2 antagonism attenuates behavioral improvements on a radial arm maze following traumatic brain injury. J Cereb Blood Flow and Metab.

23. Rafols JA, **Kreipke CW**, Mouhiddin S, Schafer PC, Michael D, Petrov T. (in press). Endothelin receptors A and B are expressed in distinct cellular compartments of rat hippocampus following global ischemia: an immunocytochemical study. Canadian Journal of Physiology and Pharmacology.

24. **Kreipke CW**, Schafer PC, Bedford C, Petrov T, Rossi NF, Rafols JA. (in press). Endothelin receptor A antagonism ameliorates hypoperfusion and improves cognitive outcome following traumatic brain injury (TBI). J Neurotrauma.

C. Research Support

Ongoing Research Support

R01 NS39860 T. Rafols (PI) 3/10/04-4/30/09
NIH-NINDS
Role: CO-I (70% effort)
"Control of microvascular tone in traumatic brain injury"
Investigates the role of endothelin receptors in the control of the microcirculation in a rat model of traumatic brain injury.

VA RR & D Award. Rossi (PI) 1/01/08-12/31/11
VA Rehabilitation
Role: CO-I (30% Effort)
"Conditioning, microvascular tone & rehabilitation post brain trauma"
Investigates the role of exercise in the control of microcirculation in a rat model of traumatic brain injury.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME Mihir Bagchi	POSITION TITLE Associate Professor
eRA COMMONS USER NAME Aa0839	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Bihar University, Ranchi, India	B.S.	1959	Biology, Chemistry
Ranchi University, Ranchi, India	M.S.	1962	Zoology
University of Vermont, Burlington, VT	Ph.D.	1969	Zoology

A. Positions and Honors

1966-1968 Graduate Assistant, University of Vermont, Burlington, VT.
1969-1972 Postdoctoral Research Associate, Oakland University, Rochester, MI.
1972-1973 Instructor, Dept. of Biological Science, Oakland University, Rochester, MI.
1973-1975 NIH Special Fellow, Kresge Eye Institute, Detroit, MI.
1975-1981 Assistant Professor, Wayne State University, Anatomy/Cell Biology, Detroit, MI.
1981-present Associate Professor, Wayne State University, Anatomy/Cell Biology, Detroit, MI.

B. Selected peer-reviewed publications (from 1985-2007) (publications total – 52)

M. Bagchi, M.J. Caporale and S.R. Gordon. The effects of hyperosmolar culture medium on the protein synthesis and morphology of epithelial cells of the cultured rabbit lens. Expt. Eye Res. 40:449-508, 1985.

M. Bagchi, M.J. Caporale, R. Wechter and H. Maisel. Vimentin synthesis by tissue and organ cultured lens epithelial cells. Expt. Eye Res. 40:385-392, 1985.

J. Heslip, M. Bagchi, S. Zhang, S. Alousi and H. Maisel. An intrinsic membrane glycoprotein of the lens. Current Eye Res. 5:958-969, 1986.

M. Bagchi, M.J. Caporale, C.V. Harding and W. Bobrowski. A study of morphology and protein synthesis of mammalian ocular lens maintained in organ culture. Lens Research 4:55-77, 1987.

M. Bagchi, A.A. Dave, A.K. Singh and M.J. Caporale. Iris-ciliary complex inhibits protein synthesis by organ cultured mammalian ocular lens cells. Invest Ophthalmol. Vis. Sci. 28:759-762, 1987.

M. Bagchi, M.J. Caporale and A.A. Dave. Role of iris-ciliary complex in the control of protein synthesis by the organ cultured rabbit ocular lens. Expt. Eye Res. 46:897-907, 1988.

B. Kiessling, R. Barrett, M.J. Caporale, L. Hazlett, and M. Bagchi. A study of experimental cataract produced by sugar alcohols in the organ cultured mammalian ocular lens. Ophthalmic Res. 20:237-244, 1988.

C.V. Harding, N.J. Unakar, M. Bagchi, L. Chylack, R. Jampel, W. Bobrowski and D. Harding. Elemental and structural studies of the rat galactose cataract. Lens Research 6:477-501, 1989.

A.K. Singh and M. Bagchi. Effect of vitreous humor on the organ cultured rabbit ocular lens. I. Protein synthesis and electrolyte balance. Lens and Toxicity Research 6:823-831, 1989.

A. Banerjee, D.M. Richiert, K. Emanual, A.K. Singh, and M. Bagchi. Studies on the possible role of vitreous humor on the protein synthesis and morphology of the organ cultured adult lens. II. Epithelial cells. Biochimica et Biophysica Acta 1076:330-336, 1991.

M. Bagchi and K. Emanuel. Effect of vitreous humor on the organ cultured rabbit ocular lens. III. Morphology and elemental analysis. Lens and Eye Toxicity Res. 8:444-467, 1991.

T.K. Miyogi, K. Emanuel, J. Parafina, and M. Bagchi. The effects of iris-ciliary complex on the organ cultured rabbit ocular lens. Lens and Eye Toxicity Res. 8(1):43-60, 1991.

A. Banerjee, D.M. Richiert and M. Bagchi. Phosphorylation of small molecular weight polypeptides in the iris-ciliary complex, aqueous humor and vitreous humor. Biochemicia et Biophysica Acta 1077:56-64, 1991.

M. Bagchi and K. Emanuel. Elemental profiles in Emory mouse lens. Lens and Eye Toxicity Res. 8(1):61-74, 1991.

A. Banerjee, K. Emanuel , J. Parafina and M. Bagchi. The mammalian iris-ciliary complex affects organization and synthesis of cytoskeletal proteins or organ and tissue cultured lens epithelial cells. J. Cell Biochem. 50:1-16, 1992.

A. Banerjee, J. Parafina, M. Bagchi. Growth regulation of mammalian ocular lens by vitreous humor. J. Cell Biochem. 49:1-8, 1992.

N.J. Unakar, W.F. Bobrowski, J.Y. Tsui, M. Bagchi, and C.V. Harding. Elemental studies in rat lens during galactose cataract reversal. Curr. Eye Res. 12(7):675-683, 1993.

M. Bagchi, A. VanWijnen, M. Katar, H. Merriman, J. Lian, J. Stein, G. Stein, and H. Maisel. Sequence-specific DNA binding activities of nuclear matrix proteins of mammalian lens epithelial cells. J. Cell Biochem. 58:1-5, 1995.

M. Bagchi, A. Roher, A. Banerjee, R. Barrett, L. Hazlett, T. Kasunic, and H. Maisel. Identification of a ubiquitin like protein in the mammalian vitreous humor. J. Cell Biochem. 61:26-31, 1996.

M. Bagchi, S. Anasari, M. Katar and H. Maisel. Non-chromatin nuclear proteins of mammalian lens epithelial cells. J. Cell Biochem. 64:644-650, 1997.

M. Bagchi, S. Ansari, D.M. Lindenmuth, A. VanWijnen, J. Lian, J. Stein and G. Stein. Nuclear matrix associated DNA binding proteins of ocular lens epithelial cells. Molecular Biology Reports. 25:13-19, 1998.

M. Bagchi, M. Katar and H. Maisel. A heat shock factor-like protein in the nucleus of tissue cultured lens epithelial cells. J. Cell Biochem. 80:382-387, 2001.

M. Bagchi, M. Ireland, M. Katar and H. Maisel. Heat shock proteins of the chicken lens. J. Cell Biochem. 82:409-414, 2001.

M. Bagchi, M. Katar and H. Maisel. Heat shock protein of adult and embryonic human ocular lenses. J. Cell Biochem. 84:278-284, 2002.

M. Bagchi and H. Maisel. Effect of exogenous stress on the tissue cultured mouse lens epithelial cell. J. Cell Biochem. 86:302-306, 2002.

M. Bagchi, M. Katar, J. Lewis and H. Maisel. Associated proteins of lens adherence junction. J. Cell Biochem. 86:700-703, 2002.

M. Bagchi, M. Katar, K. Lo, H. Maisel. Paralamimn of the chicken lens. J. Cell Biochem. 89:917-921, 2003.

M. Bagchi, M. Katar, W.-K. Lo, R. Yost, C. Hill and H. Maisel. ERM proteins of the lens. J. Cell Biochem. 92:626-630, 2004.

K.R. Badri, S. Modem, H. Gerard, I. Khan, M. Bagchi, A.R. Hudson, and T.R. Reddy. Regulation of sam 68 activity by small heat shock protein 22. J. Cell Biochem. J. Cell Bio. 99:1353-1362, 2006.

M. Bagchi, T. Petrov and H. Maisel. Lamins of ocular lens epithelial cells. J. Cell Biochem.150:923-928, 2007.

M. Bagchi, T.R. Reddy, R. Skoff, S. Modem, D.A, Bessert, and H. Maisel. Effect of thermal stress on the early and late passaged mouse lens epithelial cells. J. Cell Biochem. 102:1036-1042, 2007.

C.W. Kreipke, R. Morgan, G. Roberts, M. Bagchi and J. Rafols. Calponin phosphorylation in cerebral cortex microvessel mediates sustained vasoconstriction after traumatic brain injury. Neurol. Res. 29:369-374, 2007.

R. Morgan, C.W. Kreipke, G. Roberts, M. Bagchi and J. Rafols. Neurovascularization following traumatic brain injury, possible evidence for both angiogenesis and vasculogenesis. Neurol. Res. 29:375-381, 2007.

C. Research Support

None.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Donald M. Kuhn	Professor
eRA COMMONS USER NAME	
aa3071	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Presbyterian College	BS	1972	Biopsychology
University of South Carolina	PhD	1976	Behavioral Pharmacology
Princeton University	Postdoc	1976-1977	Electrophysiology
National Institutes of Health	Postdoc	1977-1983	Biochemical Pharmacology

A. Positions and Honors

Positions and Employment

1983-1986-Chief, Section on Biochemical Pharmacology, National Heart Lung & Blood Institute, NIH
1985-1986-Alexander von Humboldt Fellow, Department of Neurochemistry, Goethe University, Frankfurt, Germany
1987-present- Professor, Department of Psychiatry and Behavioral Neurosciences, Center for Molecular Medicine and Genetics, and Institute for Chemical Toxicology, Wayne State University School of Medicine
1993-1994-Visiting Professor, Dept. Molecular Genetics and HHMI, Univ. Texas Southwestern Medical Center, Dallas, Texas (Sabbatical leave in Dr. T. Sudhof's lab)
1998-present-Investigator, John D. Dingell VA Medical Center, Detroit, MI

Other Experience and Professional Memberships

1994-1998 Member, NIDA-C (now NMB) Scientific Review Subcommittee
1998-2002 Member, MDCN-4 Scientific Review Subcommittee
1999- Member, Editorial Board Journal of Neurochemistry
1999- Ad hoc reviewer for MDCN-3, IFCN-7, Neurological Sciences & Disorders B, NIDA Cebra Program, and numerous SEPs for NIDA, NINDS, and NIMH
2001- National Scientific Advisory Council, American Federation for Aging Research
2004- Member, Neurobiology A Merit Review Subcommittee, Dept. Veterans Affairs
2006- Member, NMB Scientific Review Subcommittee

Honors

1985- Fellow, Alexander von Humboldt Foundation

B. Selected peer-reviewed publications (in chronological order)

(Publications selected more than 135 peer-reviewed publications and book chapters)

Kuhn, D.M., Arthur, R., Jr., and Yoon, H., and Sankaran, K. Tyrosine hydroxylase in secretory granules from bovine adrenal medulla: Evidence for an integral membrane bound form. J. Biol. Chem. 265, 5780-5786, 1990.
Wolf, W.A., Zaija. E., Arthur, R.A. Jr., Anastasiadis, P.Z., Levine, R.A., and **Kuhn, D.M.** Effect of tetrahydrobiopterin on serotonin synthesis, release, and metabolism in superfused hippocampal slices. J. Neurochem. 57, 1191-1197, 1991.
Johansen, P.A., Jennings, I., Cotton, R.G.H., and **Kuhn, D.M.** Immobilization of tryptophan hydroxylase by

immune adsorption: A method to study regulation of catalysis. Brain Res. Bull. 29, 949-953, 1992.

Wolf, W.A. and **Kuhn, D.M.** Molecular pharmacology of the neuronal serotonin transporter: Role of essential sulfhydryl groups in ligand binding and transport. J. Biol. Chem. 267, 20820-20825, 1992.

Johansen, P.A., Jennings, I., Cotton, R.G.H., and **Kuhn, D.M.** Tryptophan hydroxylase is phosphorylated by protein kinase A. J. Neurochem. 65, 882-888, 1995.

Johansen, P.A., Jennings, I., Cotton, R.G.H., and **Kuhn, D.M.** Activation and phosphorylation of tryptophan hydroxylase by exogenous protein kinase A. J. Neurochem. 66, 817-823, 1996.

D'Sa, C., Arthur, R.E., Jr., States, J.C., and **Kuhn, D.M.** Tryptophan hydroxylase: Cloning and expression of the native enzyme in a mammalian cell line. J. Neurochem. 67, 900-906, 1996.

Kuhn, D.M. and Arthur, R.E., Jr. Inactivation of brain tryptophan hydroxylase by nitric oxide. J. Neurochem. 67, 1072-1077, 1996.

D'Sa, C.M., Arthur, R.A., Jr., and **Kuhn, D.M.** Expression and deletion mutagenesis of tryptophan hydroxylase fusion proteins: delineation of the enzyme catalytic core. J. Neurochem. 67, 917-926, 1996.

Kuhn, D.M. and Arthur, R.A. Inactivation of tryptophan hydroxylase by nitric oxide: Enhancement by tetrahydrobiopterin. J. Neurochem. 68, 1495-1502, 1997.

Kuhn, D.M., Arthur, R.A., and States, J.C. Phosphorylation and activation of brain tryptophan hydroxylase: Identification of serine-58 as a substrate site for protein kinase A. J. Neurochem. 68, 2220-2223, 1997.

Kuhn, D.M. and Arthur, R.A. Molecular mechanism of the inactivation of tryptophan hydroxylase by nitric oxide: Attack on critical sulfhydryls which spare the enzyme iron center. J. Neurosci.17, 7245-7251, 1997.

Kuhn, D.M. and Arthur, R.E. Dopamine inactivates tryptophan hydroxylase and forms a redox-cycling quinoprotein: Possible endogenous toxin to serotonin neurons. J. Neurosci. 18, 7111-7117, 1998.

Anastasiadis, P.Z., Imerman, B., Louie, M.C., States, J.C., **Kuhn, D.M.**, and Levine, R.A. Tetrahydrobiopterin as obligatory mediator of PC12 cell proliferation induced by EGF and NGF. Eur. J. Neurosci., 9, 1831-1837, 1997.

Kuhn, D.M., Arthur, R.E., Thomas, D.M., and Elferink, L.A. Tyrosine hydroxylase is inactivated by catechol-quinones and converted to a redox-cycling quinoprotein: Relevance to Parkinson's Disease. J. Neurochem. 73, 1309-1317, 1999.

Kuhn, D.M. and Geddes, T.J. Peroxynitrite inactivates tryptophan hydroxylase via sulfhydryl oxidation: Coincident nitration of enzyme tyrosyl residues has minimal impact on catalytic activity. J. Biol. Chem. 274, 29726-29732, 1999.

Kuhn, D.M. and Arthur, R.A. L-DOPA quinone inactivates tryptophan hydroxylase and converts the enzyme to a redox-cycling quinoprotein. Mol. Brain Res., 73, 78-84, 1999.

Kuhn, D.M., Aretha, C.W. and Geddes, T.J. Peroxynitrite inactivation of tyrosine hydroxylase: Mediation by sulfhydryl oxidation, not tyrosine nitration. J. Neuroscience, 19, 10289-10294, 1999.

Kuhn, D.M. and Geddes, T.J. Molecular footprints of neurotoxic amphetamine action. Annals NY Academy of Sciences, 914, 92-103, 2000.

Anastasiadis, P.Z., Jiang, H., Bezin, L., **Kuhn, D.M.**, and Levine, R.A. Tetrahydrobiopterin enhances apoptotic cell death following withdrawal of trophic support. J. Biol. Chem. 276, 9050-9058, 2001.

Kuhn, D.M. Dopamine and Its Modulation of Drug-Induced Neuronal Damage, E. Massaro (Ed.). In: Handbook of Neurotoxicology, Volume 2, Drugs of Abuse, Humana Press, pp 175-197, 2002.

Kuhn, D.M., Sadidi, M., Lu, X., Kriepke, C., Geddes, T., Borges, C., and Watson, J.T. Peroxynitrite-induced nitration of tyrosine hydroxylase: Identification of tyrosines 423, 428, and 432 as sites of modification by MALDI-TOF mass spectrometry and tyrosine-scanning mutagenesis. J. Biol. Chem., 277, 14336-14342, 2002.

Kuhn, D.M. and Geddes, T.J. Reduced nicotinamide nucleotides prevent nitration of tyrosine hydroxylase by peroxynitrite. Brain Research, 933, 85-89, 2002.

Park, S., Ferrer, J., Javitch, J.A., and **Kuhn, D.M.** Peroxynitrite inactivates the human dopamine transporter by modification of cysteine 342: Potential mechanism of neurotoxicity in dopamine neurons. J. Neuroscience, 22, 4399-4405, 2002.

Kuhn, D.M. Regulation of Tyrosine Hydroxylase by S-glutatiolation: Relevance to Conditions Associated with Dopamine Neuronal Damage, S. Milstien and G. Kapatos (Eds.). In: Pteridines and Folates in Biology and Medicine, Plenum Press, 61-64, 2002.

Borges, C.R., Geddes, T.J., Watson, J.T., and **Kuhn, D.M.** Dopamine biosynthesis is regulated by S-glutathionylation: Potential mechanism of tyrosine hydroxylase inhibition under oxidative stress. J. Biol. Chem., 277, 48295-48342, 2002.

Park, S.U., Geddes, T.J., Javitch, J.A., and **Kuhn, D.M.** Dopamine prevents peroxynitrite-induced nitration of

tyrosine hydroxylase by peroxynitrite and nitrogen dioxide: Is nitrotyrosine formation an early step in dopamine neuronal damage? J. Biol. Chem, 278, 28736-28742, 2003.

Borges, C.R., **Kuhn, D.M.**, and Watson, J.T. Mass mapping sites of nitration in tyrosine hydroxylase: Random versus selective nitration of three tyrosine residues. Chem. Res. Toxicol., 16, 536-540, 2003.

Kuhn, D.M. and Geddes, T.J. Tetrahydrobiopterin Prevents Nitration of Tyrosine Hydroxylase by Peroxynitrite and Nitrogen Dioxide: Implications for Nitrotyrosine as a Mediator of Dopamine Neuronal Damage. Molecular Pharmacology, 64, 946-953, 2003.

Thomas, D.M., Verbeem, D., Liu, X., and **Kuhn, D.M.** Identification of differentially regulated transcripts in mouse striatum following methamphetamine treatment: An oligonucleotide microarray approach. J. Neurochemistry, 88, 380-393, 2004.

Albertson, D.N., Pruetz, B., Schmidt, C.J., **Kuhn, D.M.**, Kapatos, G., and Bannon, M.J. Decreased Expression of Myelin-Related Genes in Human Cocaine Abusers. J. Neurochemistry, 88, 1211-1219, 2004.

Kuhn, D.M., Sakowski, S., Sadidi, M., and Geddes, T.J. Nitrotyrosine as a marker for peroxynitrite-induced neurotoxicity: The beginning or the end of the end of dopamine neurons. J. Neurochemistry, 89, 529-536, 2004.

Thomas, D.M., Dowgiert, J., Geddes, T.J., Verbeem, D., Liu, X., and **Kuhn, D.M.** Microglial activation is a pharmacologically specific marker for the neurotoxic amphetamines. Neurosci. Lett., 367, 349-354, 2004.

Thomas, D.M., Walker, P.D., Benjamins, J.A., Geddes, T.J., and **Kuhn, D.M.** Methamphetamine neurotoxicity in dopamine nerve endings of the striatum is associated with microglial activation. J. Pharmacol. Exp. Ther., 311, 1-7, 2004.

Thomas, D.M. and **Kuhn, D.M.** Attenuated microglial activation mediates tolerance to the neurotoxic effects of methamphetamine. J. Neurochem., 92, 790-797, 2005.

Thomas, D.M. and **Kuhn, D.M.** Cyclooxygenase-2 is an obligatory factor in methamphetamine-induced neurotoxicity. J. Pharmacol. Exp. Ther., 313, 870-876, 2005

Sadidi, M., Geddes, T.J., and **Kuhn, D.M.** S-thiolation of tyrosine hydroxylase by reactive nitrogen species in the presence of cysteine or glutathione. Antioxidants and Redox Signaling, 7, 863-869, 2005.

Thomas, D.M., Francescutti-Verbeem, D.M., and **Kuhn, D.M.** Gene expression profile of activated microglia under conditions associated with dopamine neuronal damage. FASEB J, (FASEB J Express article 10.1096/fj.05-4873fje. Published online December 29, 2005; link to paper via http://www.fasebj.org/cgi/reprint/05-4873fjev1?ijkey=dHQ/XZdAjW3Sg), 2005.

Sakowski, S.A., Geddes, T.J. and **Kuhn, D.M.** Mouse tryptophan hydroxylase isoform 2 and the role of proline 447 in enzyme function. J. Neurochem., 96, 758-765, 2006.

Kuhn, D.M., Francescutti-Verbeem, D., and Thomas, D.M. Dopamine quinones cause microglial activation and induce a neurotoxic gene expression profile: relationship to methamphetamine-induced nerve ending damage. Annals of the New York Academy of Sciences, 1074, 31-41, 2006.

Sakowski, S.A., Geddes, T.J., Thomas, D.M. and **Kuhn, D.M.** Differential tissue distribution of tryptophan hydroxylase isoforms 1 and 2 as revealed with monospecific antibodies. Brain Research, 1085, 11-18, 2006.

Thomas, D.M., Francescutti-Verbeem, D.M., and **Kuhn, D.M.** Gene expression profile of activated microglia under conditions associated with dopamine neuronal damage. FASEB J (FJ Express Summary), 20, 515-517, 2006.

Kuhn, D.M., Sakowski, S.A., Geddes, T.J., Wilkerson, C., and Haycock, J.W. Phosphorylation and activation of tryptophan hydroxylase 2: Identification of serine-19 as the substrate site for calcium-dependent protein kinase II. J. Neurochem., 103, 1567-1573, 2007.

Thomas, D.M., Francescutti-Verbeem, D.M., and **Kuhn, D.M.** The newly synthesized pool of dopamine determines the severity of methamphetamine-induced neurotoxicity. J. Neurochem., 605-616, 2008.

Kuhn, D.M., Francescutti-Verbeem, D.M., and Thomas, D.M. Dopamine disposition in the presynaptic process regulates the severity of methamphetamine-induced neurotoxicity. Ann. N.Y. Acad. Sci., in press, 2008.

Ni, W., Geddes, T.J., Priestley, J.R., Szasz, T., **Kuhn, D.M.**, and Watts, S.W. The existence of a local serotonergic system in rat peripheral arteries. Brit. J. Pharmacol., in press, 2008.

Linder, A.E., Ni, W., Szasz, T., Burnett, R., Diaz, J., Geddes, T.J., **Kuhn, D.M.**, and Watts, S.W. A serotonergic system in veins: serotonin-transporter independent uptake. J. Pharmacol. Exp. Ther., in press, 2008.

Thomas, D.M., Francescutti-Verbeem, D.M., and **Kuhn, D.M.** Methamphetamine-induced neurotoxicity and microglial activation are not mediated by fractalkine receptor signaling. J. Neurochem. in press, 2008.

Research Support

Ongoing (Active) Research Support

NIH/NIDA 5 R01 DA10756 04/10/07-04/09/12
Neurotoxic Amphetamines, Radicals, and 5HT Neurons
The major goal of the study is to determine the mechanisms by which neurotoxic amphetamine-derived reactive oxygen and nitrogen species alter function of dopamine and serotonin neurons through their effects on important phenotypic marker proteins in these neuronal elements.
Role: PI

NIH/NIDA 1 RO1 DA017327 04/01/05 – 03/30/10
Methamphetamine Neurotoxicity and Microglial Activation
The goal of this project is to elucidate the role of microglia in the neurotoxic effects associated with methamphetamine and other neurotoxic amphetamines. When funded, the budget of DA017327 was reduced by 25% (arbitrary) and the decision of which projects could be completed was left to the discretion of the PI. Because this area (neurotoxic amphetamines/microglia) has received considerable interest in our lab and in a growing number of others, and considering that this represents nearly the entire thrust of our lab, the some of the studies that had to be cut from DA017327 are now part of DA10756 (this application).
Role: PI

Department of Veterans Affairs Merit Award 03/15/07-03/14/11
Brain Injury by Blast Overpressure: Role of Microglial Activation
The goal of this project is to characterize microglial involvement in brain damage caused by blast overpressure. We have developed a model of blast overpressure, a form of traumatic brain injury, that allows testing of cultured cells and brain slices.
Role: PI

Projects completed in the past 3 years

NIH/NIDA 1 K05 DA14692 10/05/02-12/04/07
Molecular Biology of Drug Abuse
This is a senior scientist career development award.
Role: PI

NIH/NIDA 1 T32 DA07310 07/01/00-06/30/06
Neuroscience Training in Drug Abuse
This is a training grant that supports two predoctoral and two postdoctoral fellows. This training program is in hiatus temporarily. Our department experienced some significant changes in faculty re-assignment to other academic units, and several other key investigators on the T32 have left Wayne State. Therefore, we are re-configuring this training program as the Translational Neuroscience Program to reflect more accurately the current mentoring and research expertise of our departmental faculty. We anticipate submission of a renewal application in March 2007.
Role: PI

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
E. Mark Haacke, Ph.D.	Director
eRA COMMONS USER NAME: ak5444	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Toronto	B.S.	1973	Mathematics & Physics
University of Toronto	M.S.	1975	Theoretical Physics
University of Toronto	Ph.D.	1978	High Energy Physics

NOTE: The Biographical Sketch may not exceed four pages. Items A and B (together) may not exceed two of the four-page limit. Follow the formats and instructions on the attached sample.

A. Positions and Honors.

POSITIONS

1981-1983	**Research Geophysicist**, projects seismic tomography, scattering theory and imaging, Gulf Research and Development, Pittsburgh, PA
1983-1985	**Senior Research Scientist**, projects included NMR sequence development, fast imaging, reduction of motion artifacts, chemical shift imaging, new reconstruction schemes, Picker International, Highland Heights, OH
1983-1985	**Lecturer in Physics**, developed a new course on MRI, Case Western Reserve University, Cleveland, OH
1985-1989	**Assistant Professor of Radiology and Physics**, Head, MR Physics and Basic Science. Developed flow, motion and fast imaging techniques as well as new high resolution, high S/N reconstruction techniques, Case Western Reserve University
1989-1993	**Associate Professor**, in the Department of Radiology with joint appointments in Physics and Biomedical Engineering, Case Western Reserve University
1993-1999	**Professor of Radiology**, Director MR Imaging Research, Mallinckrodt Institute of Radiology, Washington University, St. Louis, MO
1999-present	**Director**, The Magnetic Resonance Imaging Institute for Biomedical Research, St. Louis, MO
2002-present	**Professor of Radiology**, Wayne State University, Detroit, MI

HONORS

1975	Ontario Graduate Scholarship
1976	Ontario Graduate Scholarship
1977	E.F. Burton Fellowship
1989	Sylvia Sorken Greenfield Award for the best paper in Medical Physics
1992	Fellow of the Society Award for the Society of Magnetic Resonance Imaging
1994	Silver Medal Award, Society of Magnetic Resonance
1997	Poster Award at the 14th Annual Meeting, European Society for Magnetic Resonance in Medicine and Biology. J.R. Reichenbach, E.M. Haacke, B.C.P. Lee, Ch. Przetak, W.A. Kaiser
1998	Marie-Sklodowska-Curie Prize for Visualization of Cerebral Venous Structures Using High Resolution MRI by J.R. Reichenbach, L.R. Schad, M. Essig, E.M. Haacke, W.A. Kaiser
2000	Poster Prize of the XXVI Congress of the European Society of Neuroradiology 2000. J.R. Reichenbach, L. Jonetz-Mentzel, C. Fitzek, H.-J. Mentzel, E.M. Haacke, W.A. Kaiser.
2002	Scientific Exhibition Award ECR 2002 Cum Laude. J.R. Reichenbach, C. Fitzek, L. Jonetz-Mentzel, D. Sauner, H.-J. Mentzel, E.M. Haacke, W.A. Kaiser. European Congress of Radiology
2004	Gold Medal Award, International Society of Magnetic Resonance in Medicine

B. Selected peer-reviewed publications (in chronological order).
(Publications selected from 163 peer-reviewed publications)

1. F.G.C. Hoogenraad, P.J.W. Pouwels, M.B.M. Hofman, S.A.R.B. Rombouts, C. Lavini, M.O. Leach, E.M. Haacke. High-resolution Segmented EPI in a Motor Task fMRI Study. Magnetic Resonance Imaging 2000; 18:405-409.
2. E.H. Haacke, Z-P. Liang. Challenges of Imaging Structure and Function with MRI. IEEE Eng Med Biol 2000;19(5):55-62.
3. Y. Wang, Y. Yu, D. Li, K.T. Bae, J.J. Brown, W. Lin, E.M. Haacke. Artery and Vein Separation Using Susceptibility Dependent Phase in Contrast-Enhanced MRA. JMRI 2000; 12:661-670.
4. J.R. Reichenbach, M. Barth, E.M. Haacke, M. Klarhöfer, W.A. Kaiser, E. Moser. High-Resolution MR Venography at 3.0 Tesla. J Comp Assist Tomog 2000; 24(6): 949-957.
5. F.G.C. Hoogenraad, P.J. W. Pouwels, M.B.M. Hofman, J.R. Reichenbach, M. Sprenger, E.M. Haacke. Quantitative Differentiation Between BOLD Models in fMRI. MRM 2001; 45:233-246.
6. Y-C.N. Cheng, E.M. Haacke, Y-J. Vu. An Exact Form for the Magnetic Field Density of States for a Dipole. MRI 2001; 19:1017-1023.
7. D. Li, J. Zheng, H.J. Weinmann, K.T. Bae, E.M. Haacke. Contrast-enhanced MRI of Coronary Arteries: Comparison of Intravascular and Extravascular Contrast Agents and Technical Consideration. Radiology 2001; 218:670-678.
8. K. Scheffler, E. Seifritz, D. Bilecen, R. Venkatesan, J. Hennig, M. Deimling, E.M. Haacke. Detection of BOLD Changes by Means of a Frequency-sensitive TrueFISP Technique: Preliminary Results. NMR in Biomedicine 2001; 14:490-496.
9. Yu-Chung N. Cheng, E. Mark Haacke. Predicting BOLD signal changes as a function of blood volume fraction and resolution. NMR in Biomedicine 2001;14:468-477.
10. K. Gurleyik, E. Mark Haacke. Quantification of Errors in Volume Measurements of the Caudate Nucleus Using Magnetic Resonance Imaging. JMRI 2002;15:353-363.
11. E.M. Haacke, G. Herigault, Y. Yu, D. Kido, K. Tong, A. Obenaus, J. Reichenbach. Observing Tumor Vascularity Noninvasively Using Magnetic Resonance Imaging. Image Anal Stereol 2002;21:107-113.
12. Tong, K.A., Ashwal, S., Holshouser, B.A., Shutter, L., Herigault, G., Haacke, E.M., Kido, D.K. Hemorrhagic Shearing Lesions in Children and Adolescents with Posttraumatic Diffuse Axonal Injury: Improved Detection and Initial Results. Radiology 2003; 227:332–339.
13. Tong, K.A., Ashwal, S., Holshouser, B.A., Shutter, L., Herigault, G., Haacke, E.M., Kido, D.K. Improved Detection of Hemorrhagic Shearing Lesions in Children with Post-traumatic Diffuse Axonal Injury – In Children: Clinical Correlation with Hemorrhagic Lesions. Ann Neurol 2004;56:36–50.
14. N.D. Wycliffe, J. Choe, B. Holshouser, U.E. Oyoyo, E.M. Haacke and D. Kido. Reliability in Detection of Hemorrhage in Acute Stroke by a New Three-Dimensional Gradient Recalled Echo Susceptibility-Weighted Imaging Technique Compared to Computed Tomography: A Retrospective Study. JMRI 2004;20:372-377.
15. R.K. Singh, V.S., Deshpande, E.M. Haacke, S.M. Shea, Y. Xu, R.M. McCarthy, J. Carr, and D. Li. Coronary Artery Imaging Using Three-Dimensional Breath-Hold Steady-State Free Precession with Two-Dimensional Iterative Partial Fourier Reconstruction. JMRI 2004;19:645-649.
16. E.M. Haacke, Y. Xu, Y.C.N. Cheng, J. Reichenbach. Susceptibility Weighted Imaging (SWI). MRM 2004;52:612-618.
17. D. Haddar, E.M. Haacke, V. Sehgal, Z. DelProposto, G. Salamon, O. Seror, N. Sellier. L'imagerie de susceptibilite magnetique: theorie et applications. J Radiol 2004;85;1901-1908.
18. A. Ruascher, J. Sedlacik, M. Barth, E.M. Haacke, J.R. Reichenbach. Non-invasive Assessment of Vascular Architecture and During Modulated Blood Oxygenation Using Susceptibility Weighted MRI (SWI). (In review at MRM – Dec 2004).
19. V. Sehgal, Z. Delproposto, D. Haddar, E.M. Haacke, A.E. Sloan, L.J. Zamorano, G. Barger, J. Hu, Y. Xu, K.P. Prabhakaran, I.R. Elangovan, J. Neelavalli, J.R. Reichenbach. Susceptibility Weighted Imaging of Brain Masses. (In review at JMRI – Dec 2004).
20. E.M. Haacke, N.Y.C. Cheng, M.J. House, Q. Liu, J. Neelavalli, R.J. Ogg, A. Khan, M. Ayaz, W. Kirsch, A. Obenaus. Imaging Iron Store in the Brain Using Magnetic Resonance Imaging. MRI 2005; 23;1–25.
21. J. Hu, Y. Xia, W. Feng, Y. Xuan, Y. Shen, E. M. Haacke, Q. Jiang. Orientational dependence of trimethyl ammonium signal in human muscles by 1H magnetic resonance spectroscopic imaging. MRI 2005; 23;97–104.

22. Shen Y, Kou Z, Kreipke CW, Petrov T, Hu J, Haacke EM. 2006. In vivo measurement of tissue damage, oxygen saturation changes and blood flow changes after experimental traumatic brain injury in rats using susceptibility-weighted imaging. Magn Reson Imaging 25(2):219-227.

C. Research Support.

1. R01 AG20948 — Kirsch, Wolff – PI (Subcontract with Loma Linda University, California)
   BRP 10/1/2002-9/30/2007
   Iron Metabolism Alteration in Alzheimer 's Disease

   Goals: Evaluate the role of IRP-2 in Alzheimer'sdisease and the role of a new MRI technique to image brain iron. — Role: Co-Investigator

2. Siemens Medical Solutions — Haacke, E.Mark – PI
   Master Research Agreement
   7/1/01 – 6/30/06

   Goals: Collect clinical data for SWI in the areas of trauma, stroke, and vascular disease. — Role: PI

3. R01 NS038292 — Jiang, Quan – PI (Subcontract with Henry Ford Hospital, Michigan)
   NINDS 07/01/04 – 06/30/08
   MRI and SVZ Cell Therapy for Severe Stroke

   Goals: Develop noninvasive in vivo MRI methodology for tracking transplanted MSCs and their effects on the host brain. — Role: Co-Investigator

Ongoing Research Support

VA RR & D Award. Rossi (PI) 1/01/08-12/31/11
VA Rehabilitation
Conditioning, microvascular tone & rehabilitation post brain trauma
CO-I (20% Effort)

NIH-NINDS RO1 NS39860 (PI) 06/01/04-05/31/09
Control of Microvascular tone in Traumatic Brain Injury.
Co-I

NIH-NINDS RO1 NS044100 (CO-I) 07/01/03-06/30/08
The Unfolded Protein Response after Brain Ischemia.
Co-I

Ongoing Research Support

R01 NS39860 J Rafols (PI) 3/10/04-4/30/09
NIH-NINDS
Role: CO-I (70% effort)
"Control of microvascular tone in traumatic brain injury"
Investigates the role of endothelin receptors in the control of the microcirculation in a rat model of traumatic brain injury.

VA RR&D Award 1/01/08-12/31/11
VA Rehabilitation Award
Role: CO-I (30% effort)
"Conditioning, microvascular tone & rehabilitation"
Investigates the role of exercise in controlling microcirculation after traumatic brain injury.

Research Support for Dr. Kuhn

Ongoing (Active) Research Support

NIH/NIDA 1 K05 DA14692 10/05/02-10/04/08
Molecular Biology of Drug Abuse
This is a senior scientist career development award
Role: PI

NIH/NIDA 1 RO1 DA017327-01 04/01/05 – 03/30/10
Methamphetamine Neurotoxicity and Microglial Activation
The goal of this project is to elucidate the role of microglia in the neurotoxic effects associated with methamphetamine and other neurotoxic amphetamines.
Role: PI

Research Support for Dr. Haacke.

1. R01 AG20948 Kirsch, Wolff – PI (Subcontract with Loma Linda University, California)
   BRP 10/1/2002-9/30/2008
   Iron Metabolism Alteration in Alzheimer 's Disease
   Role: Co-Investigator
   Goals: Evaluate the role of IRP-2 in Alzheimer's disease and the role of a new MRI technique to image brain iron.

2. R01 NS038292 Jiang, Quan – PI (Subcontract with Henry Ford Hospital, Michigan)
   NINDS 07/01/04 – 06/30/08
   MRI and SVZ Cell Therapy for Severe Stroke
   Role: Co-Investigator
   Goals: Develop noninvasive in vivo MRI methodology for tracking transplanted MSCs and their effects on the host brain.

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

1. Project Director / Principal Investigator (PD/PI)

Prefix: Dr.
* First Name: Jose
Middle Name: A.
* Last Name: Rafols
Suffix: Ph.D.

* New Investigator? ☒ No ☐ Yes

Degrees: PhD

2. Human Subjects

Clinical Trial? ☒ No ☐ Yes

* Agency-Defined Phase III Clinical Trial? ☐ No ☐ Yes

3. Applicant Organization Contact

Person to be contacted on matters involving this application

Prefix: Ms.
* First Name: Gail
Middle Name:
* Last Name: Ryan
Suffix:

* Phone Number: 313.577.2294
Fax Number: 313.577.2653
Email: orspsmail@wayne.edu

* Title: Senior Director

* Street1: 5057 Woodward
Street2: 13th Floor
* City: Detroit
County: Wayne
* State: MI: Michigan
Province:
* Country: USA: UNITED STATES
* Zip / Postal Code: 48202

PHS 398 Cover Page Supplement

4. Human Embryonic Stem Cells

* Does the proposed project involve human embryonic stem cells? ☒ No ☐ Yes

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://stemcells.nih.gov/registry/index.asp. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

Cell Line(s): ☐ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

PHS 398 Modular Budget, Periods 1 and 2

OMB Number: 0925-0001

Budget Period: 1

Reset Entries | Start Date: 05/01/2010 | End Date: 04/30/2011

A. Direct Costs

	* Funds Requested ($)
* Direct Cost less Consortium F&A	250,000.00
Consortium F&A	
* Total Direct Costs	250,000.00

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	Modified Total Indirect Costs	52		130,000.00
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number) U.S. Department of Education, Richard Dowd, (312) 886-6503

Indirect Cost Rate Agreement Date 03/30/2005

Total Indirect Costs 130,000.00

C. Total Direct and Indirect Costs (A + B)

Funds Requested ($) 380,000.00

Budget Period: 2

Reset Entries | Start Date: 05/01/2011 | End Date: 04/30/2012

A. Direct Costs

	* Funds Requested ($)
* Direct Cost less Consortium F&A	250,000.00
Consortium F&A	
* Total Direct Costs	250,000.00

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	Modified Total Indirect Costs	52		130,000.00
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number) U.S. Department of Education, Richard Dowd, (312) 886-6503

Indirect Cost Rate Agreement Date 03/30/2005

Total Indirect Costs 130,000.00

C. Total Direct and Indirect Costs (A + B)

Funds Requested ($) 380,000.00

PHS 398 Modular Budget, Periods 3 and 4

Budget Period: 3

Reset Entries | Start Date: 05/01/2012 | End Date: 04/30/2013

A. Direct Costs

	* Funds Requested ($)
* Direct Cost less Consortium F&A	250,000.00
Consortium F&A	
* Total Direct Costs	250,000.00

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	Modified total indirect costs	52		130,000.00
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number): U.S. Department of Education, Richard Dowd, (312) 886-6503

Indirect Cost Rate Agreement Date: 03/30/2005

Total Indirect Costs: 130,000.00

C. Total Direct and Indirect Costs (A + B)

Funds Requested ($): 380,000.00

Budget Period: 4

Reset Entries | Start Date: 05/01/2013 | End Date: 04/30/2014

A. Direct Costs

	* Funds Requested ($)
* Direct Cost less Consortium F&A	250,000.00
Consortium F&A	
* Total Direct Costs	250,000.00

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	Modified total indirect costs	52		130,000.00
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number): U.S. Department of Education, Richard Dowd, (312) 886-6503

Indirect Cost Rate Agreement Date: 03/30/2005

Total Indirect Costs: 130,000.00

C. Total Direct and Indirect Costs (A + B)

Funds Requested ($): 380,000.00

PHS 398 Modular Budget, Periods 5 and Cumulative

Budget Period: 5

Reset Entries | Start Date: 05/01/2014 | End Date: 04/30/2015

A. Direct Costs

	* Funds Requested ($)
* Direct Cost less Consortium F&A	250,000.00
Consortium F&A	
* Total Direct Costs	250,000.00

B. Indirect Costs

	Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1.	Modified total indirect costs	52		130,000.00
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number): U.S. Department of Education, Richard Dowd, (312) 886-6503

Indirect Cost Rate Agreement Date: 03/30/2005

Total Indirect Costs: 130,000.00

C. Total Direct and Indirect Costs (A + B)

Funds Requested ($): 380,000.00

Cumulative Budget Information

1. Total Costs, Entire Project Period

*Section A, Total Direct Cost less Consortium F&A for Entire Project Period	$ 1,250,000.00
Section A, Total Consortium F&A for Entire Project Period	$
*Section A, Total Direct Costs for Entire Project Period	$ 1,250,000.00
*Section B, Total Indirect Costs for Entire Project Period	$ 650,000.00
*Section C, Total Direct and Indirect Costs (A+B) for Entire Project Period	$ 1,900,000.00

2. Budget Justifications

Personnel Justification	1249-Personnel.pdf	Add Attachment	Delete Attachment	View Attachment
Consortium Justification		Add Attachment	Delete Attachment	View Attachment
Additional Narrative Justification		Add Attachment	Delete Attachment	View Attachment

Personnel

Jose Rafols, Ph.D., Principal Investigator (3.6 cal. mos.) has over thirty years of experience performing studies of brain ischemia and TBI. He was the PI of the original grant successfully funded in 2000. In November of 2006, with full approval of NIH-NINDS, Dr. Rafols reassumed PI status with the sudden illness of Dr. Petrov (former PI). Dr. Rafols will conduct histopathologic and ICC analyses. He will direct and be responsible for facilitating the research design. He will assume responsibility that all experiments are conducted in a proper manner and finalized in a timely fashion. In addition, he will be responsible for the final preparation of manuscripts and grants that directly accompany this work.

Christian Kreipke, Ph.D., CO-Investigator (8.4 cal. mos.) is an emerging investigator in the field of TBI research. He joined Dr. Rafols' laboratory to conduct brain trauma research over three years ago and, hence has finished his post-doctoral training. During the current lab funding, he has enhanced the laboratory's goals by including behavioral analysis and added to the pharmacological experiments designed. Dr. Kreipke has experience in mentoring students, working with the model of brain injury, molecular biology and in assessing animal behavior. He will assist Dr. Rafols in leading the direction of the research. In conjunction, Dr. Kreipke will personally conduct many of the experiments and will oversee training of student assistant.

Mihir Bagchi, Ph.D., CO-Investigator (2.4 cal. mos.) has over thirty years of experience and a wealth of publications in conducting molecular and biochemical experiments. In the past year, as evidenced in our list of manuscripts in the current funding, Dr. Bagchi has already begun working with us to fill the void left by the sudden illness of Dr. Petrov. He will oversee and personally conduct many of the Western blots and 2-dimensional gels as needed.

Srinivasu Kallakuri, Graduate Student (6 cal. mos.) is completing his PhD dissertation in our laboratory on the effects of ETrA and B after TBI. Since 50% of his salary and tuition are covered by the Graduate Program, we are requesting to supplement 50% of his salary and tuition. He has published in our laboratory on ETrA and B localization following TBI and will be instrumental in producing TBI, ICV injections, and ICC for ETrA and B.

Patrick Schafer, Research technician (12 cal. mos.) will be instrumental in carrying out immunocytochemistry and Western analysis. He is also trained in surgery and behavioral analysis. He has currently published in the laboratory. Further, one initiative within this proposal is the training of students in neurosciences. This training will be overseen by Dr. Kreipke.

Steven Schafer, Research technician (12 cal. mos.) will be instrumental in carrying out the behavioral analysis. As evidenced in the proposal, much of our behavioral work is labor intensive and is done 20-30 days consecutively, as well as is conducted during evening hours to correspond with the active cycle of rat's diurnal cycle. Therefore, we feel a dedicated person to this task who will not have to conduct experiments during day hours is needed. Further, one initiative within this proposal is the training of students in neurosciences. This training will be overseen by Dr. Kreipke.

Donald Kuhn, Ph.D., (Consultant) will provide expertise and access to RT-PCR and in pharmacological treatments (see letter of support). No salary is requested.

E. Mark Haack, Ph.D., (Consultant) will provide expertise and access to equipment for carrying out MRI analyses (see letter of support). No salary is requested.

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OMB Number: 0925-0001

PHS 398 Research Plan

1. Application Type:

From SF 424 (R&R) Cover Page and PHS398 Checklist. The responses provided on these pages, regarding the type of application being submitted, are repeated for your reference, as you attach the appropriate sections of the research plan.

*Type of Application:

☐ New ☒ Resubmission ☐ Renewal ☐ Continuation ☐ Revision

2. Research Plan Attachments:

Please attach applicable sections of the research plan, below.

1. Introduction to Application (for RESUBMISSION or REVISION only)	1250-Introduction.pdf	Add Attachment	Delete Attachment	View Attachment
2. Specific Aims	1251-Specific AIMS A2.pdf	Add Attachment	Delete Attachment	View Attachment
3. Background and Significance	1252-Background and Signifi	Add Attachment	Delete Attachment	View Attachment
4. Preliminary Studies / Progress Report	1253-Progress report and Pr	Add Attachment	Delete Attachment	View Attachment
5. Research Design and Methods	1254-Research Design and Me	Add Attachment	Delete Attachment	View Attachment
6. Inclusion Enrollment Report		Add Attachment	Delete Attachment	View Attachment
7. Progress Report Publication List	1255-Progress report public	Add Attachment	Delete Attachment	View Attachment

Human Subjects Sections

Attachments 8-11 apply only when you have answered "yes" to the question "are human subjects involved" on the R&R Other Project Information Form. In this case, attachments 8-11 may be required, and you are encouraged to consult the Application guide instructions and/or the specific Funding Opportunity Announcement to determine which sections must be submitted with this application.

8. Protection of Human Subjects		Add Attachment	Delete Attachment	View Attachment
9. Inclusion of Women and Minorities		Add Attachment	Delete Attachment	View Attachment
10. Targeted/Planned Enrollment		Add Attachment	Delete Attachment	View Attachment
11. Inclusion of Children		Add Attachment	Delete Attachment	View Attachment

Other Research Plan Sections

12. Vertebrate Animals	1256-Vertebrate Animals.pdf	Add Attachment	Delete Attachment	View Attachment
13. Select Agent Research		Add Attachment	Delete Attachment	View Attachment
14. Multiple PI Leadership Plan		Add Attachment	Delete Attachment	View Attachment
15. Consortium/Contractual Arrangements		Add Attachment	Delete Attachment	View Attachment
16. Letters of Support		Add Attachment	Delete Attachment	View Attachment
17. Resource Sharing Plan(s)		Add Attachment	Delete Attachment	View Attachment

18. Appendix | Add Attachments | Remove Attachments | View Attachments

General comment. We would like to thank the review panel once again for their overall enthusiasm and positive review of the previous grant proposal, as well as for their helpful comments on how to increase the merit of this final resubmission to make it worthy of funding. We were encouraged by statements such as, "Overall, this project is highly significant" and "…the application has an important translation component". Due to the shared enthusiasm for pursuing this investigation, we have proceeded with new preliminary data addressing the major concerns that were raised by the individual reviewers and are confident that with these additions the review panel will find this proposal meritorious and worthy of funding. Due to the fact that the only perceived major weakness was shared by all three reviewers we will first address this issue. Next we will address the minor concerns raised by each individual reviewer.

NOTE: All changes in the grant proposal are indicated by italics.

Overall concern: "A major concern with this project relates to its ability to address the underlying mechanisms involved.
While the overall goal of this proposal is to provide a novel avenue for developing new strategies for improving outcome following TBI and while all reviewers agree that, based on our preliminary data, we have satisfied this objective, we do acknowledge that in the former proposal we still did not fully address the mechanism by which this treatment may be acting. Therefore, we greatly appreciate the review panel's willingness to offer their expertise in how to address this important issue. SPECIFIC AIM 2 is a newly designed AIM to further address the mechanism. SPECIFIC AIM 5 tests the hypothesis that improved CBF after TBI is mediated by ETrA antagonist dependent upregulation of ETrB, thus allowing for vasodilation. It was stated by reviewer 1 that, "it would be important to conduct this aim using not only TBI but also using the direct ET-1 infusion…this would add significant enthusiasm for the application". Reviewer 3 recapitulated this idea. Therefore, upon the reviewers recommendation we have performed this experiment in a limited number of animals as included as new preliminary data. Surprisingly, ET-1 infusion, alone, did not produce the upregulation in ETrB, suggesting that, indeed, TBI and not hypoperfusion, alone, causes a regulatory change in the way that ETrB is expressed following ETrA antagonist delivery. While beyond the scope of this proposal, future work in our laboratory will be aimed at elucidating the reason why TBI causes receptor expression changes.

Reviewer 1.
1. Perhaps, the investigator should consider increasing CBF to normal rates by a mechanism other than ET-1 related mechanism after TBI and also after administration of ET-1…Such a strategy would separate CBF effects of ET-1 with other effects not related to CBF. In this way, the investigator would be able to determine if the detrimental effects of ET-1 is through reductions in CBF or via another mechanism.
We completely agree that this is an important line of investigations. To that end we have developed a novel method to increase CBF independent of ET-1 (newly added AIM 2). As published in a recent review in Journal of Cellular and Molecular Medicine (Kreipke and Rafols, 2009) we have obtained an antibody which blocks the phosphorylation of calponin (Cp), an important contractile protein. By blocking Cp phosphorylation, we have shown that we can block the hypoperfusion following TBI (newly added preliminary data). Furthermore, we have shown that by blocking hypoperfusion in this manner we were able to improve histopathological and behavioral outcome following TBI. Taken together, these results suggest that CBF changes following TBI are causing negative outcomes.

2. Do the authors know if the dose of BQ-788 was effecting blocking at a dose of 10 ng? There needs to be some means to determine if the receptors are being blocked.
We also agree with this point. To that end we have repeated studies in AIM 4 to include a higher dose (50nmol) of BQ-788. At this dose, CBF is significantly decreased beyond that of TBI alone at 24 and 48 hours post TBI. Furthermore, we have included new preliminary data included in AIM 5 which shows that while blood flow is increased (i.e., hypoperfusion ameliorated) following TBI+ injection of the ETrA antagonist as compared to TBI, alone, when BQ-788 is added blood flow is once again decreased. This suggests that BQ-788 does have action on CBF in the presence of the ETrA-mediated upregulation of ETrB.

Reviewer 2.
1. Overall, the outcome studies will be strengthened with the addition of histopathological analyses.
We completely agree with the reviewer. Therefore we have added FluoroJade labeling as a measure of cellular injury to all studies. Many of these measures were conducted in a small number of animals and are included as new preliminary data.

Reviewer 3.
Reviewer 3 recapitulated the concerns from reviewers 1 and 2.

A. SPECIFIC AIMS

Traumatic brain injury (TBI) is reportedly the leading cause of death and disability among children and young adults (CDC Report, 2004). TBI is also known as the signature injury in the War on Terrorism. USA Today reported (2007) that 83% of brain injured marines and sailors returning from Iraq suffer cognitive impairments. The combined emotional and financial costs of civilian casualties and those associated with the military as they try to rejoin civilian life is overwhelming. Therefore, there is a compelling need to implement effective therapies to improve the quality of life of those suffering from TBI.

Among multiple sequelae, TBI results in three major pathologies: cerebral edema which leads to a critical rise in intracranial pressure, diffuse axonal injury which brings about disruption of neural circuits underlying cognitive and motoric behaviors, and alterations in cerebral blood flow (CBF) that cause a persistent state of hypoperfusion and improper delivery of vital metabolites to neural tissue. While all three pathologies combine to cause substantial morbidity and mortality seen in the clinical setting, this laboratory has been successfully funded for the last decade to focus on hypoperfusion following TBI. The driving hypothesis for this work has been that: **Restoration of a dysfunctional CBF after TBI leads to improved histopathologic and cognitive outcomes after injury**.

In the first funding period, using a rodent model of brain trauma our laboratory established that endothelin-1, a powerful vasoconstrictor, is upregulated up to 48 hours post TBI. Inhibiting the synthesis of ET-1 ameliorates this hypoperfusion. In the current funding period we also published that ET receptors, ETrA and B, are also dysregulated following TBI, resulting in increased synthesis and protein expression of ETrA directly after TBI and increased synthesis and expression of ETrB 24 hours after trauma. Blockade of ETrA activation either through protein inhibition or the selective receptor antagonist, BQ-123, prior to injury improved cerebral blood flow (CBF) following TBI. Blockade of ETrB had no effect. Taken together, these data suggest that there is a correlation between ET-1 upregulation following TBI and hypoperfusion. However, what is not yet known is whether ET-1 upregulation causes the observed changes in rat cerebral perfusion, microvessel morphology, and endothelin (ET)-1 receptors (rA and rB). Furthermore, how this may translate into functional, behavioral deficits is unknown. In addition, the effects of ETrA and B antagonism directly following injury have not been investigated. Finally, the effects of clinically available drugs aimed at antagonizing the ETrA receptor have never been studied following TBI. Therefore, in order to provide a logical extension to the previous funding periods, to address the gaps in our knowledge thus far, and to begin translating this work to the clinical setting, we have developed the following central hypothesis: **blockade of upregulated ET-1 signaling after TBI improves the deficits in cerebral blood flow as well as histopathologic and cognitive outcome resulting from injury.** 5 AIMS for this competing renewal are designed to test this central hypothesis:

SPECIFIC AIM 1 tests the hypothesis that alterations in rat cerebral perfusion, microvessel morphology, and endothelin (ET)-1 receptors (rA and rB) occurring after TBI are caused by ET-1 upregulation and that these changes contribute to histophathology and impaired spatial learning following injury.

SPECIFIC AIM 2 test the hypothesis that TBI-mediated hypoperfusion exacerbates cell injury and contributes to behavioral deficits following head trauma.

SPECIFIC AIM 3 tests the hypothesis that intracerebroventricular (ICV) or IV injections of the ETrA selective antagonist BQ-123 a recently introduced ETrA antagonist Clazosentan, which is undergoing Phase II clinical trial for prevention of cerebral vasospasm after stroke directly following TBI ameliorates hypoperfusion of brain and improves cognitive outcome.

SPECIFIC AIM 4 tests the hypothesis that ICV or IV injections of the ETrB antagonist BQ-788 or mixed ETrA/B antagonist, Bosentan, directly following TBI had no effect on hypoperfusion and thus does not improve cognitive outcome following TBI.

SPECIFIC AIM 5 tests the hypothesis that improved CBF after TBI is mediated by ETrA antagonist-dependent upregulation of ETrB, thus allowing for vasodilation.

Our multidisciplinary approach will define a mechanism by which trauma affects the cellular components of the microvasculature and provide a rational basis for therapeutic interventions that target specific blockade of endothelin receptors in the CNS in an attempt to improve the dysfunctional microcirculation and cognitive deficits following TBI.

B. BACKGROUND AND SIGNIFICANCE

Traumatic Brain Injury (TBI) and Brain Pathology: Why focus on endothelin pharmacology?

TBI results in several major histopathologic events, including among others: cerebral edema which leads to a critical rise in intracranial pressure, diffuse axonal injury which brings about disruption of neural circuits underlying cognitive and motoric behaviors, and alterations in the brain's microcirculation that cause a persistent state of hypoperfusion and improper delivery of vital metabolites to neural tissue which in turn exacerbates neuronal injury leading to secondary injuries. In closed head TBI incidents in humans, all these events are thought to significantly contribute to the ensuing morbidity and mortality encountered in clinical settings. The experiments designed in the present proposal address the issue of the dysfunctional microcirculation after TBI, focusing on the role of the peptide endothelin (ET)-1 and its receptors (rA and rB) before and after injury. While ET-1 receptor antagonism is widely used therapeutically in clinical trials involving kidney, lung and heart pathologies, a similar approach to improve vasospasm after stroke or trauma remains to be implemented. Using a well validated, closed head acceleration impact rodent model, our laboratory has demonstrated a key role of ET-1 and its receptors in causing the state of chronic hypoperfusion after TBI. In the present proposal we address the mechanistic underpinnings of the ET-1 receptors in the non-injured and injured brain and provide insights into novel pharmacological therapies that may be translated from laboratory bench into the clinical setting.

Endothelin (ET) - 1

During the previous funding period we have established that alterations in ET-1 metabolism after TBI are causally related to the sustained hypoperfusion affecting the brain microcirculation. Endothelins (ETs) are peptides which at high concentrations exert an extremely potent and long-lasting vasoconstriction (ET-1 vasoconstrictive effects are 10X greater than those of angiotensin-1); yet, when present at physiologically low levels, may elicit vasodilation (Warner et al., 1994b). The genes that encode for ETs are present in human, porcine, murine and rat cells (Inoiue et al., 1989; Saida et al., 1989). ETs are expressed in a variety of tissues: neurons, adrenal gland, lung, heart and kidney (Masaki, 1993), as well as endothelial cells of porcine aorta from which they were first isolated (Yanagisawa et al., 1988). Synthesis of ETs has been shown in astrocytes and macrophages (Hori et al., 2001; Petrov et al., 2002a).

There are three isoforms of the peptide, ET-1, ET-2 and ET-3, each containing 21 amino acids, 2 disulfide bridges and 1 hydrophobic C-terminal end with a tryptophan in position 21 (Saida et al., 1989; Yanagisawa and Masaki, 1989). Despite their structural similarity, ET-1 and ET-2 are almost equally potent, while ET-3 is less potent (Zimmermann and Seifert, 1998). The ETs also have different affinities to their receptors, i.e., the affinity of ETrA to ET-1 and ET-2 is two orders of magnitude higher than that to ET-3; ETrB, on the other hand, shows similar affinity to all three isoforms (Sakurai et al., 1992). Furthermore, when ETs bind to their receptors they dissociate very slowly indicating a long lasting effect (Hirata et al., 1988; Marsault et al, 1993). We have shown that there is a characteristic shift in ETs receptor cellular localization in brain after trauma (Kallakuri et al., 2007a and b) implying these receptors in mechanisms may relate not only to microvascular responses but also, as suggested by others (Zimmermann and Seifert, 1998), to the development of secondary brain injury.

Endothelin Receptors

The endothelin receptors belong to the superfamily of G-protein-coupled heptahelical receptors and activate phospholipase C (PLC) (Arai et al., 1990; Sakurai et al., 1990). They have a molecular weight of approximately 47 kDa and contain seven transmembrane domains of 20-27 hydrophobic amino acid residues (Warner et al, 1994b). In general, ETs contribute to the development of chronic vasospasm via activation of both receptors (Shigeno et al, 1995) (detailed below).

ETrA. This receptor has been detected in vascular smooth muscle (SM) in brain blood vessels (Hori et al., 1992), neurons (Kurokawa et al., 1997; Kallakuri et al, 2007a), astrocytes and endothelial cells (Nakagomi et al., 2000). When ETs are released they bind to ETrA on SM. At the time of ET-1 release from the endothelium, an intermediate form of the peptide, called big-ET, is also released. Big-ET is converted to ET-1 by endothelin-converting enzymes (ECEs) on the surface of SM (D'Orleans-Juste et al., 1990; McMahon et al., 1991), and this ET-1 binds also to ETrA in SM. The resulting SM contraction is Ca^{2+}-dependent and may be mediated in part by activation of PLC (Povlishock et al., 1983) and phosphorylation of contractile proteins in SM such as calponin (Kreipke et al., 2006;2007,a,c).

Alterations in the expression of ETrA were observed in several brain pathological conditions. mRNA for ETrA was significantly upregulated 3 and 7 days following subarachnoid hemorrhage (Itoh et al., 1993) or after kainic acid-induced brain damage (Sakurai-Yamashita, 1997). We have published that ETrA mRNA as well as protein are

upregulated during the hypoperfusion phase brought about by TBI (Kreipke and Petrov 2005; Kallakuri et al., 2007a, b; Kreipke et al., in press).

Effects of ETrA blockade. BQ-123 (cyclo-D-α-aspartyl-L-prolyl-D-valyl-L-leucyl-D-tryptophyl) has been shown to have a high affinity for ETrA and a potent action in attenuating the contractile function of ETrA (Ihara et al., 1992; Ishikawa et al., 1992). It has a high affinity for ETrA and, even at higher doses, does not affect body temperature, body weight, mean blood pressure or heart rate (Hirose et al., 1995). In addition it has a prolonged effect (days) after a single ICV injection (Hirose et al., 1995; Josko et al., 2001; Yip and Krukoff, 2002). For these reasons BQ-123 has been suggested as a potential therapeutic agent for improving blood flow after brain trauma.

Hypertension associated with increased contractility of blood vessels in spontaneously hypertensive rats was attenuated by systemic application of BQ-123 (Morel and Godfraind, 1994). ET-1-induced hypertension was also attenuated significantly following blockade with this antagonist (Warner et al, 1994a). In addition, the increase in arterial pressure observed following restraint stress was reduced by ICV application of BQ-123 (Yip and Krukoff, 2002). In the heart following ischemia/hypoperfusion, BQ-123 effectively antagonized the coronary constrictive effect of ET-1 and improved functional recovery during reperfusion (Han et al., 1995). Application of this antagonist was also protective in ischemic acute renal failure in rats (Mino et al., 1992).

Attenuation of ETrA in brain resulted generally in an increase in the cross-sectional diameter of cerebral arteries, especially following subarachnoid hemorrhage (Ishikawa et al, 1994; Warner et al, 1994b). In vivo blockade of ETrA with BQ-123 ameliorated the outcome of autoimmune encephalomyelitis, possibly by reducing the hypoperfusion observed in this disease (Shin et al., 2001). Intracisternal application of BQ-123 abolished the reduced cerebral blood flow (CBF) induced by subarachnoid hemorrhage (Clozel and Watanabe, 1993). Blockade of ETrA with this antagonist also led to normalization of neurological performance and CBF within two day post cardiac arrest (Krep et al., 2000). In addition, in combination with acetylcholine, application of BQ-123 induced a widespread significant increase (in some cases up to 50%) in CBF (Granstam et al., 1998). Blockade of ETrA resulted in reduction of the lesion due to attenuated vasoconstriction following cold injury to the brain (Gorlach et al., 2001) or focal stroke (Barone et al, 2000).

In the current funding period we sought to test the effects of ETrA blockade on microvascular tone following TBI. We previously showed that an ICV injection of 40μg BQ-123 prior to TBI was able to ameliorate the TBI-induced hypoperfusion (Kreipke et al., 2007, abstract for ET-10). We also showed that ETrA antagonism reversed other TBI-induced effects such as morphological and biochemical alterations in contractile proteins in vascular SM (e.g., calponin upregulation and phosphorylation) which inhibited the mechanism of SM contraction (Kreipke et al, 2007, abstract for Brain 07), thus inducing vasodilation and improved cerebral blood flow.

ETrB. Of the two receptors, ETrB is the more abundant in brain tissue (Hama et al., 1997). It has been detected in astrocytes (MacCumber et al., 1990; Kallakuri et al., 2007), neurons (Nakagomi et al., 2000), microglia Sakurai-Yamashita et al., 1997) endothelial cells and vascular SM (Peters et al., 2001; Kallakuri et al., 2007). Activation of ETrB triggers events similar to those described during activation of ETrA. More specifically, activation of ETrB causes increase in cytosolic Ca^{2+}, stimulation of extracellularly-regulated kinase pathways (Marsault et al., 1990; Lazarini et al., 1996) and modulation of cytoskeletal actin organization (Cazaubon et al., 1997; Koyama and Baba, 1996). ETrB is distributed to selectively vulnerable areas of the CNS such as the hippocampus and cerebral cortex (Bousso-Mittler et al., 1989; Kloog and Sokolovsky, 1989; Williams et al., 1991; Kallakuri et al, 2007). Moreover, distribution receptor binding studies after subarachnoid hemorrhage have shown a shift of receptor distribution from ETrA to ETrB (Roux et al., 1995). We have shown that TBI results in an upregulation of ETrB 24 hours following injury, this upregulation occurring after the initial increase in ETrA (Kreipke et al., in press).

The effect of ETrB activation on microvascular tone is somewhat controversial. It has been reported to act as both a vasodilator (Randall et al., 1989; Hasunuma et al., 1990; Fukuroda et al., 1994; Ivy et al., 1994; Sato et al., 1995) and a vasoconstrictor (Clozel et al., 1992; Harrison et al., 1992; Moreland et al., 1992; Teerlink et al., 1994). While it has been suggested that, due to this disparity, two subtypes, ETrB1 and ETrB2 of ETrB exist, only subtypes ETrA and B have been cloned (Cazaboun and Courand, 1998; Nakagomi et al., 2000; Ho et al., 2001). In endothelium, ETrB is thought to mediate vasodilation through ET-1 clearing and nitric oxide (NO) release (reviewed in Pollack and Schneider, 2006). The activity of ETrB may be related to its localization. In endothelium, ETrB is thought to mediate vasodilation through ET-1 clearing and nitric oxide (NO) release (reviewed in Pollack and Schneider, 2006). However, in one study it was shown that ETrB exerts a vasoconstrictive force within pulmonary SM (Perreault and Baribeau, 1995). Another study showed that an initial dose of 1μmol of BQ-3020 applied directly to pial arterioles resulted in vasodilation while subsequent doses in the same vessel given 10 min apart resulted in no effect followed by vasoconstriction (Touzani et al., 1997).

Principal Investigator/Program Director (Last, First, Middle): Rafols, Jose A.

Effects of ETrB blockade. ETrB antagonism, like ETrB function, is somewhat controversial. BQ-788 has been shown to attenuate increased perfusion resistance in tumor (HSN fibroblastoma) vasculature (Bell et al., 1999). In another study elevation of blood pressure induced by ET-1 application was eliminated by BQ-788 (Ishikawa et al., 1994). Several other studies, however, have shown that ETrB antagonism has no effect on blood pressure (reviewed in Pollack and Schneider, 2006). One study showed that, in pial vessels, BQ-788 abolished BQ-3020 (a selective ETrB agonist)-induced vasodilation (Touzani et al., 1997).

ET receptor antagonism and clinical therapeutics. Beginning in the early 1990s, endothelin was studied in humans for its potential role in the clinical setting (Vierhapper et al., 1990; Baldys-Waligorska and Szybinski, 1992). Since then, endothelin has been a target for studying a host a pathological states that include disruption of blood flow, including hypertension (Baldys-Waligorska and Szybinski, 1993), hepatorenal syndrome (reviewed in Epstein, 1994), heart failure (Sakai et al., 1996) and decreased cerebral blood flow and hypoxia (Therkelsen et al., 1994). In 1995 Luscher and Wenzel published one of the first reviews which characterized ET-antagonists as potential clinical therapeutics for vascular disorders (Luscher and Wenzel, 1995). In 1999, Benigni and Remuzzi published a follow-up which summarized data from pre-clinical and clinical studies which showed promise for specific ETrA antagonists in controlling hypertension. Bosentan, a mixed antagonist (ETrA and B) was discussed and clinical trial suggested that the potential opposing effects of ETrA and B may render Bosentan less effective (Benigni and Remuzzi, 1999). In 2003, it was reported that, after thorough investigation of ongoing clinical trial, Bosentan had some success in control of pulmonary arterial hypertension, however was not more effective than other, non-endothelial specific drugs (Krum and Liew, 2003). Once again, this may be attributed to Bosentan being a mixed antagonist. At the 2007 10th international symposium on endothelin (ET-10) in Bergamo, Italy, several investigators pointed out that while mixed antagonists have had some effects in pre-clinical studies, overall these agents have had little to no effect in the clinical setting. Therefore, it was proposed that specific ETrA antagonists may be more useful.

The first report on a new drug, produced by Actelion Pharmaceuticals, INC in Switzerland, Ro 61-1790 [5-methyl-pyridine-2-sulfonic acid 6-(2-hydroxy-ethoxy)-5-(2-methoxy-phenoxy)-2-(2-1H-tetrazol-5-yl-+ ++pyri din-4-yl)-pyrimidin-4-ylamide] was published in 1997 (Roux et al., 1997). It was found to be 1000-fold more selective for ETrA than ETrB. It was suggested that Ro 61-1790, which was renamed Clazosentan, may be useful for TBI (Sato and Noble, 1998), ischemia (Dawson et al., 1999), and subarachnoid hemorrhage (Gorlach et al., 2001). In 2006, clazosentan was included in a clinical trial to prevent vasospasm following hemorrhage (Uhlmann, 2006). Interestingly, this drug has been shown to have little effect in non-brain areas (Vuurmans et al., 2004). Therefore, selective ETrA antagonism provides a great potential for controlling vascular disruption following TBI. *Therefore, AIMS 3 and 5 of the present study address the potential benefits of ETrA antagonism following TBI: 1) In AIM 3 we will use IV injections of the selective ETrA antagonist, BQ-123 and compare these results with the novel ETrA antagonist, Clazosentan and 2) in AIM 5 we will determine a potential mechanism by which ETrA antagonism could improve outcome following TBI.*

C. PROGRESS REPORT AND PRELIMINARY DATA

This is a competitive renewal application of a project which was initially reviewed in November 2003 by the Brain Disorders and Clinical Neuroscience Study Section (ZRG1 BDNC 1 Review Group). The present progress report covers the last 4 years of the current funding cycle (March 1, 2004-January 31, 2008). Since we have decided to submit this competitive renewal one year in advance, it is expected that the final year of the current grant will be dedicated to finalizing manuscripts that currently exist as abstracts in order to complete all of the stated goals of the previous funding period.

Specific AIMS of the previous application

The overall hypothesis of the previous funding period was that, **TBI damages the microvascular endothelium, smooth muscle (SM) cells and the surrounding neuropil by modifying their ability to synthesize ETrA and ETrB.** To support this central hypothesis, we posited and subsequently tested three hypotheses which served as the independent AIMS. These were: 1) TBI causes structural and functional changes in brain microvessels (defined here as reacting arterioles and capillaries), including increased microvascular tone and upregulation of ETrA and B (protein and mRNA) which are temporally associated with diminished cerebral perfusion (i.e., hypoperfusion); 2) the TBI-induced hypoperfusion will show a trend towards normalization when the genes that encode for either ETrA or ETrB are suppressed; and 3) the sustained hypoperfusion post-TBI will improve by selective pharmacological blockage of ETrA or ETrB.

In these studies we inflicted TBI by using the Marmarou's acceleration impact model of closed head trauma. This model consists of dropping a weight (450 g from 2 m) onto a stainless steel helmet affixed to the exposed skull of male Sprague-Dawley rats (350-400g). During the previous funding period, this model underwent some scrutiny by us as well as other investigators, as to its efficacy in TBI research. Issues were raised as to whether the concussive brain injuries resulting from the model closely were reliably reproduced. Further, others suggested that the model produced too much variability between trials. To address these issues, during the previous funding period we conducted a series of trial experiments which led to substantial modifications of the model. These included: 1) drilling small holes along the length of the Plexiglas tube to practically eliminate air resistance, 2) careful alignment of the weight with the skull helmet prior to impact in order to minimize bilateral non-homogeneous effects of impact, and 3) replacement of the foam bed after each trial to maximize head acceleration. After implementation of these modifications, both histopathologic (i.e., diffuse axonal injury) and cerebral blood flow measures (by anterior spin labeling-magnetic resonance imaging, ASL-MRI) data validated the reliability of the model. After completing this preliminary study, subsequent data was generated and published in a special edition of Neurological Research on Diffuse Traumatic Brain Injury: Therapeutic Implications, vol. 29, number 4 (Rafols, 2007). In tandem with this work, we also proceeded to carry out the specific aims of the previous grant submission.

<u>Brief description of AIM 1 studies</u>

Microvessel structure and CBF. AIM 1 sought to determine microvascular alterations following TBI including, morphological changes in microvessels as determined by electron microscopy, changes in luminal diameter, changes in CBF, altered expression of ETrA and B receptors, and changes in contractile properties of endothelium and smooth muscle (SM) cells. First we observed ultrastructural alterations in the lumen and wall of sensorimotor cortical (smCx) microvessels, including evidence of enhanced SM cell contractility, endothelial cell distortion and luminal collapse, from 1 hr post TBI through 48 hr (Rafols et al., 2007). We also measured a nearly 40% reduction in the average microvascular luminal area by 4 hours with a trend to recover (21%) by 48 hours post trauma. These changes coincided temporally with a 37% reduction in cortical CBF which was first seen at 4 hr and persisted to 48 hr post TBI (Rafols et al., 2007). We initially measured CBF using laser Doppler flowmetry (LDF), but subsequently published data using ASL-MRI showing the same effect in smCx but also in deeper brain structures (e.g., thalamus and hippocampus) (Shen et al., 2007).

Stress response. We also observed the induction of brain endothelial HSP-70 stress response which coincided with endothelial and pericyte death after TBI (DeGracia et al., 2007). This study showed for the first time using TUNEL labeling the presence of a small subset of endothelial cell death and putative pericyte cell death which temporally coincided with sustained hypoperfusion and ultrastructural changes in the endothelium.

Brain ETrA and ETrB synthesis and cellular localization. We have recently performed real-time RT PCR analysis of both ETrA and ETrB and have shown that, compared to sham-operated controls, there is a trend towards an increase in ETrA gene expression at 1 hour post TBI with a significant increase at 4 hours, this increase being sustained up to 48 hours post TBI. ETrB gene expression was also slightly elevated by 4 hours post injury and remained elevated through 48 hours post trauma (Kreipke et al, in press). ETrA and ETrB protein was also significantly altered following TBI. Compared to sham-operated controls, Western analysis revealed a significant

increase in ETrA expression by 4 hr followed by normalization by 24 hr. ETrB expression gradually increased after TBI, being most prominent at 24 hr. By 48 hr, there was a trend towards normalization (Kreipke et al, in press). Immunocytochemistry revealed that, compared to sham control, ETrA was most prominent in the wall of microvessels (both SM and endothelium) at 4 hr and in cortical pyramidal neurons by 24 and 48 hr post-TBI. In contrast ETrB immunoreactivity was most pronounced at 24 hr, being localized to endothelial cells, perivascular neurons, SM and pericytes (Kallakuri et al., 2007). The post-TBI cellular localization of ETrA and ETrB was more carefully analyzed in a subsequent study using double-label immunofluorescence which revealed intense expression of ETrA in endothelium, SM, and neurons, as well as limited expression in astroglia. In contrast ETrB was expressed in endothelium and SM. It was most prominent in neurons and absent in oligodendroglia (Kallakuri et al., 2007, abstract, Society for Neuroscience annual meeting, San Diego, CA, USA).

Vascular contractile proteins in normal and injured brain. In an attempt to understand the molecular and biochemical underpinning of enhanced contractility, we conducted a subset of experiments related to a novel finding that contractile and cytoskeletal proteins are altered during SM contraction. Specifically, we found that both the contractile protein calponin (Cp) and the cytoskeletal protein caldesmon (Cd) are upregulated within cortical and hippocampal vascular SM cells and endothelium following TBI, this upregulation coinciding temporally with the TBI-induced hypoperfusion (Kreipke et al., 2006). We also observed that Cp also migrated from the luminal to the abluminal face of SM (Kreipke et al. 2007c). Additionally we published that Cp is phosphorylated directly after TBI and remains in the phosphorylated state up to 48 hr post TBI (Kreipke et al., 2007a). Given that in vitro data suggests that normally Cp phosphorylation and migration is necessary to facilitate SM contraction (Winder and Walsh, 1990; Barany et al., 1992; Worth et al., 2001), we hypothesized that Cp migration and persistent phosphorylation mediate sustained vasoconstriction after brain trauma (Kreipke et al., 2007a). On-going studies are designed to further explore this mechanism.

<u>Brief description of AIM 2 studies</u>

Antisense gene suppression of ETrA or ETrB. AIM 2 sought to determine whether prior intracerebroventricular (ICV) injection of ETrA or ETrB antisense oligodeoxynucleotides (ODNs) has an effect on CBF following TBI. In order to first establish that ODNs are effective as in vivo translational inhibitors, we measured ETrA protein levels in smCx in both control animals and in those subjected to TBI. We showed that while ODNs have no effect in normal brain, these probes were able to significantly reduce ETrA expression following TBI (Petrov et al., 2005, 2007 abstracts for International Symposia on Endothelin, ET-9 and 10, respectively). We also showed that a prior ICV injection of ETrA ODNs resulted in a partial block of the TBI-induced hypoperfusion (i.e., a CBF reduced to approximately 25% of control baseline as compared to nearly 35%). Furthermore, contrary to our hypothesis, ETrB ODNs had no effect on CBF following TBI.

<u>Brief description of AIM 3 studies</u>

Effect of ET selective receptor blockade on CBF and vasoconstriction. AIM 3 sought to determine whether a prior injection of ETrA or ETrB selective antagonists (BQ-123 and BQ-788, respectively) have an effect on CBF following TBI. We showed that a prior injection of 40μg BQ-123 ameliorates hypoperfusion following TBI. We also showed that a prior injection of 10ng BQ-788 has no effect on TBI-induced hypoperfusion (Kreipke et al., 2007, abstract for ET-10). We also showed that ETrA antagonism blocks Calponin (Cp) upregulation and phosphorylation (Kreipke et al, 2007, abstract for Brain 07). Data generated from these studies is currently being prepared for publication in JCBFM.

Taken together, these data suggest that TBI results in significant damage to the cells of the brain's microcirculation, these alterations temporally coinciding with the TBI-induced period of hypoperfusion. While upregulation of both ETrA and ETrB occurs following TBI, we show that selective ETrA blockade, either at the gene level or using a selective antagonist, BQ-123, prior to injury can reduce the extent and duration of hypoperfusion following injury. ETrB blockade prior to injury has little to no effect on the TBI-induced hypoperfusion.

<u>The Next Step</u>

Therefore, while we have a significant amount of data to support the original central hypothesis that **TBI damages the microvascular endothelium, smooth muscle cells and the surrounding neuropil by modifying their ability to synthesize ETrA and B**, there are several questions that still remain. While in the first funding period we published that TBI results in increased brain ET-1 (Petrov et al., 2002) and that this increase temporally coincides with sustained hypoperfusion, microvascular alterations and ETrA and B expression changes, a question that remains unanswered is whether an increase in brain ET-1, alone, is sufficient to induce the observed changes

in CBF, enhanced microvascular tone and ETrA and B expression. This question is addressed in AIM 1 of the current proposal.

Second, while it is important to understand the molecular and biochemical underpinnings of sustained hypoperfusion following TBI and whether this can be reversed, the functional consequences of reduced CBF following TBI are not aims of the currently funded grant. Further, whether reversal of hypoperfusion following TBI has any functional or behavioral consequence has not been tested. This proposal addresses not only CBF as an outcome, but each AIM will include in tandem behavioral and histopathologic assessments resulting from ET receptor manipulation. In order to establish an effective means of measuring behavioral outcome, during this funding period we have incorporated the automated radial arm maze into our experiments. We have successfully demonstrated spatial learning and decision making deficits following TBI as measured by the radial arm maze (Kreipke et al., 2007b). Subsequently, we have shown that ETrA blockade using an ICV injection of BQ-123 24 hr prior to TBI results in improved behavioral outcome as compared to TBI alone (Kreipke et al., 2007, abstract for ET-10). Further, ETrB antagonism (10ng BQ-788) resulted in a worsening of performance on the radial arm maze following TBI (Kreipke et al., 2007, abstract for ET-10).

Finally, while pre-injection of ETrA and ETrB ODNs and antagonists provided insights into the mechanism underlying enhanced microvascular tone after TBI, this approach is not practical and cost effective for translation into the clinical setting. Therefore all experiments in the current competing renewal are designed to test the effects of pharmacological manipulation of ETrA and B post TBI.

PRELIMINARY STUDIES PERTINENT TO THE PROPOSED AIMS IN THE CURRENT PROPOSAL

SPECIFIC AIM 1 tests the hypothesis that alterations in rat cerebral perfusion, microvessel morphology, and endothelin (ET)-1 receptors (rA and rB) occurring after TBI are caused by ET-1 upregulation and that these changes contribute to histopathology and impaired spatial learning following injury.

Levels of ET-1 in smCx and Hipp following TBI.

In order to determine an effective dose of ET-1 that may mimic the effects seen following TBI, we measured ET-1 levels in smCx and Hipp (N=6) using ELISA prior to TBI and 4, 24 and 48h post TBI.

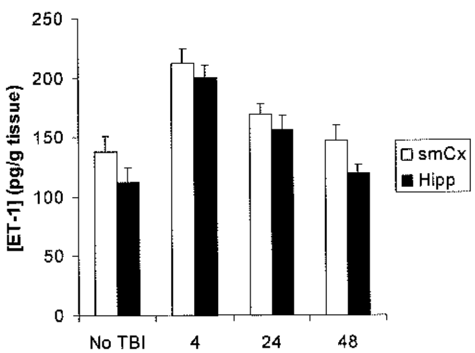

Figure 1. Levels of Et-1 in smCx and Hipp following TBI. Following TBI, ET-1 levels raised from approximately 138 pg/g in smCx and 112 pg/g in Hipp to over 200pg/g in both areas at 4 hours post TBI. Therefore, for subsequent studies we chose a dose response of 100, 200 and 400pg injection.

Effect of ET-1 combined with selective antagonism of its receptors, A or B on cerebral blood flow (CBF).

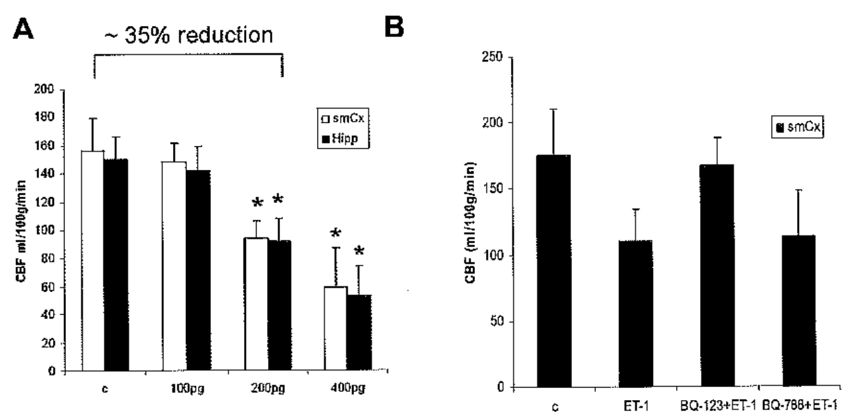

Figure 2. Effect of ET-1 alone or combined with selective antagonism of its receptors on CBF. In **A**, animals (n=4 per group) were given bilateral intracerebroventricular (ICV) injections of ET-1 (0, 100, 200 and 400pg) delivered in 10μl total volume 0.9% saline (vehicle). 1 hour after injection, CBF was measured using anterior spin labeling-magnetic resonance imaging (ASL-MRI). A dose of 40ng resulted in approximately 35%

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reduction in CBF in sensorimotor cortex (smCx) and dorsal hippocampus (Hipp). In **B**, animals (n=3 per group) were given bilateral ICV injection of vehicle (C), ET-1 (200pg), BQ-123 (a selective ETrA antagonist,10μg) followed by ET-1 (200pg) given 1 hour later, or BQ-788 (a selective ETrB antagonist, 50ng) followed 1 hour later by ET-1 (200pg). 1 hour following final injection, CBF was determined using ASL-MRI. While BQ-123 was able to ameliorate the ET-1 induced decrease in CBF, BQ-788 had no effect.

Effect of ET-1 ICV injection on ETrA expression.

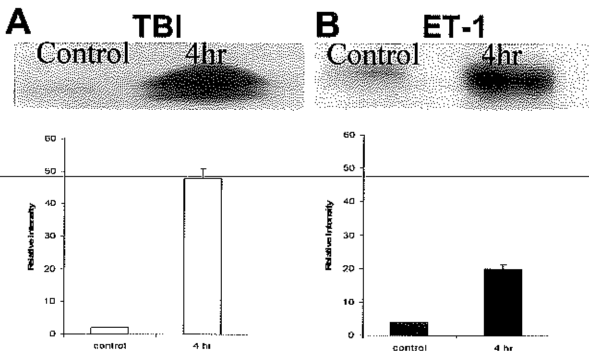


Figure 3. Comparison of the effect of TBI and ET-1 on ETrA protein expression. A) 3 animals were subjected to sham surgery (control) or to TBI (4 hr) and 4 hours after surgery smCx tissues were harvested and pooled for Western analysis of ETrA expression. TBI significantly enhanced ETrA expression (compare immunoblot in upper panels with semiquantitative optical densitometric analysis in lower panels). **B)** In another set of animals, 3 were given ICV injection of vehicle (control) and 3 were given ICV injection of 200pg ET-1 (4hr). 4 hours after injection, ET-1, similar to TBI, greatly enhanced ETrA expression. All Westerns were run twice.

Effect of ET-1 ICV injection on cognitive outcome.

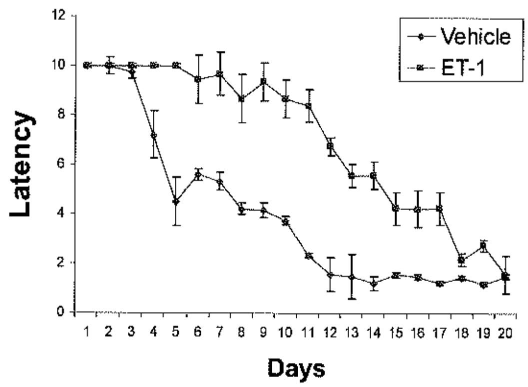
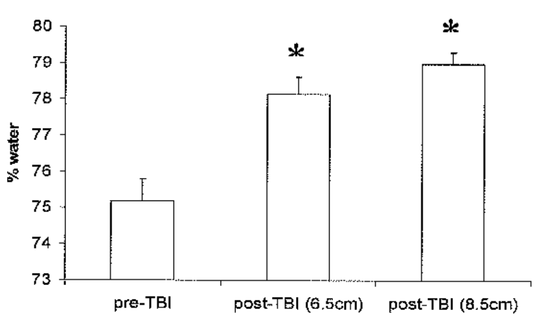


Figure 4. Effect of ET-1 on performance on a radial arm maze. Animals (N=7 per group) were either given ICV injections of vehicle (blue) or 200pg of ET-1 (pink). ET-1 increased the time it took to retrieve all pellets from the radial arm maze. A trend to achieve normal latency appeared to occur around 2-wk of injection time. For more detailed description of the behavioral testing see General Methods.

Effect of decreased ET-1 expression (40% of normal achieved using a well-characterized ET-1$^{+/-}$ heterozygous knockout model) on hypoperfusion following TBI.
In order to study whether ET-1 causes increased vasoreactivity and hypoperfusion following TBI, we have added an experiment utilizing a well-characterized heterozygous ET-1 knockout mouse (see Kuwaki et al. 1997 for review), which will be subjected to TBI. While a complete ET-1 knockout would have been preferred, such knockout is not viable due to pronounced respiratory distress (Kuwaki et al., 1997). In order to accomplish this experiment, we first characterized E. Shohami's closed head mouse model of diffuse TBI (Chen et al, 1997), fashioned after Marmarou's rat model or the model used elsewhere in this proposal. To validate this mouse model we aimed to determine whether edema, diffuse axonal injury, and decreased CBF also occurred after impact.



5a. From its inception, Marmarou's rat closed head acceleration impact model (1994) has been known to generate both significant cerebral edema and increased intracranial pressure. Therefore using Shohami's model, we measured brain water content (BWC) using a standard wet/dry method (Ding et al., 2001) to ascertain if edema resulting from impact TBI occurred also in this model. When a 95g weight was suspended from heights of both 6.5cm and 8.5cm and allowed to drop directly onto the skull midline of a 35g mouse, a significant increase in BWC was generated indicating edema formation, a hallmark pathology of TBI.

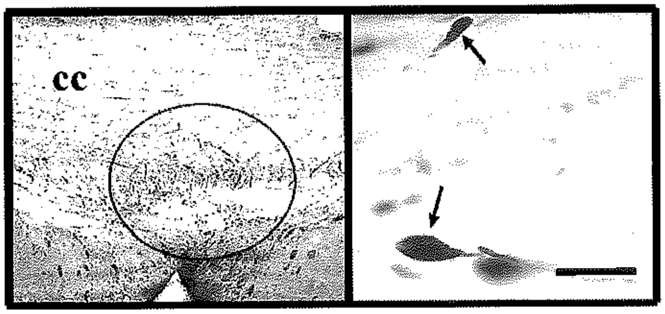


Figure 5b. Evidence of axonal retraction bulbs (as detected using β-APP staining; arrows in blowup of left panel) in corpus callosum (cc) indicates the presence of diffuse axonal injury (DAI) in the mouse model. Here we show that if a 95g weight is suspended from a height of 8.5cm and allowed to fall onto the skull midline, DAI, a second hallmark of TBI occurs. No retraction bulbs were visualized using the 6.5cm height. We have previously published such results using Marmarou's model (Rafols et al., 2007) suggesting that the pathologies in mouse and rat after TBI are similar. Measurement bar = 5μm

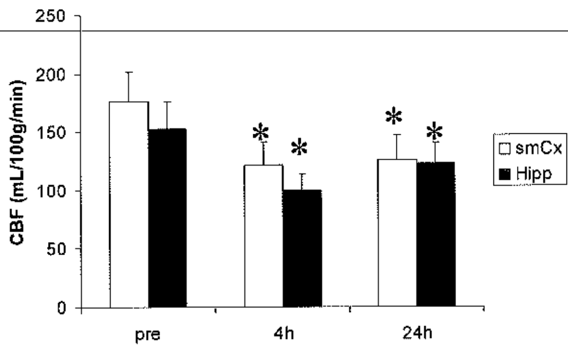


Figure 5c. Evidence of decreased CBF after TBI using Shohami's mouse model further validates the mouse model. Following a 95g weight drop from a height of 8.5cm onto the skull midline, we measured CBF using ASL-MRI. Results show a significant reduction in CBF at 4 and 24 hrs after impact which is similar to those previously described in rat (Rafols et al, 2007).

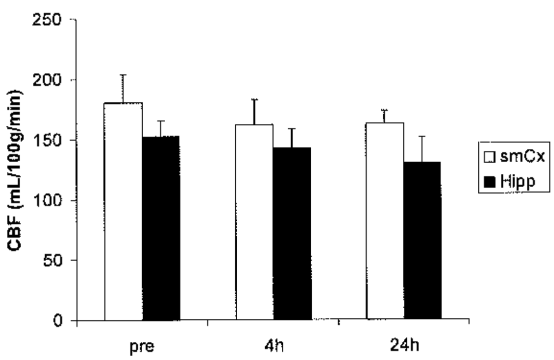


Figure 6. The effect of reduced ET-1 levels on CBF following TBI. Here we used an ET-$1^{+/-}$ knockout mouse model that is known to express only 40% of wild type ET-1 levels (Kuwaki et al., 1997). While pre-TBI CBF of heterozygous mice was similar to that of wild type animals (see Figure 6), when both groups are subjected to TBI, there was only a slight decrease in CBF of heterozygous mice at both 4 and 24h post TBI, compared to that of wild type mice at the same time periods (figure 6). Therefore if ET-1 levels are decreased (ie, knockout condition), so, too, is the extent of hypoperfusion. By inference this suggests that increased ET-1 following TBI causes hypoperfusion.

AIM 2. To test the hypothesis that TBI-mediated hypoperfusion exacerbates cell injury and contributes to behavioral deficits following head trauma.

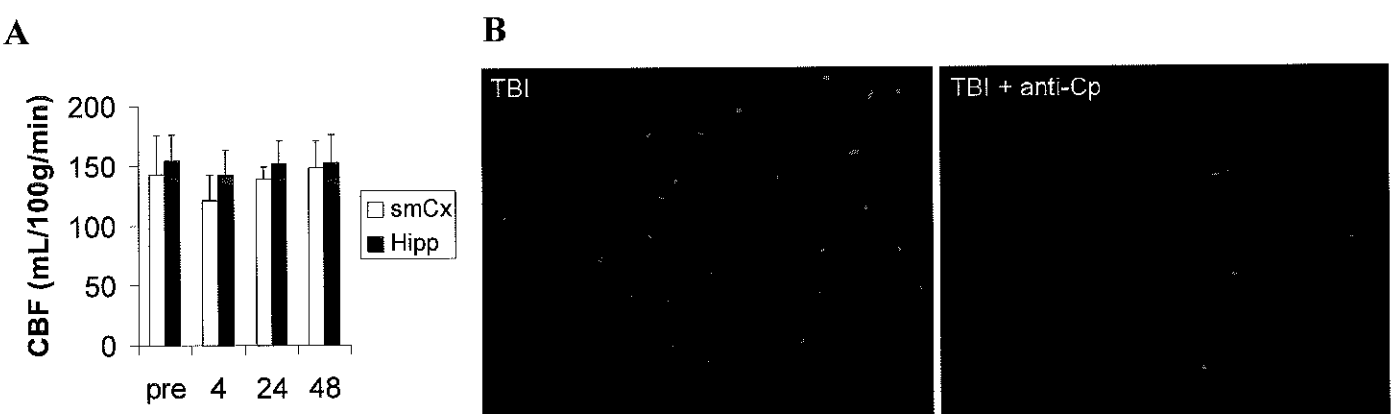


Figure 8. Effect of anti-Cp, which has been shown to block TBI-induced hypoperfusion, on extent of cell injury. *First, CBF measurements were taken in the same regions as used for determination of the effect of the ETrA antagonist (smCx and Hipp) in 3 animals prior to injection or TBI (pre) (A). Next, they were given ICV injection of 20nmol of a peptide*

generated to bind to Cp at its THR184 phosphorylation site. 1 hour later, TBI was induced. CBF was then re-measured at 4, 24 and 48 hours to test whether the antibody could block hypoperfusion. After it was determined that it could block the hypoperfusion (A), another set of rats received the same injection and then TBI was induced. 4 hours after TBI, animals were sacrificed and tissue sections containing smCx were processed for FluoroJade along side sections from animals receiving only TBI (B). Results indicate that blocking hypoperfusion using anti-Cp sufficiently reduced FJ cell labeling in layers II-III of smCx, and thus, the extent of cell injury.

AIM 3. To test the hypothesis that ICV injection of the ETrA selective antagonist BQ-123 shortly after TBI will ameliorate hypoperfusion and improve cognitive outcome following TBI.

Effect of ICV injection of BQ-123 at 30 min following TBI on CBF.

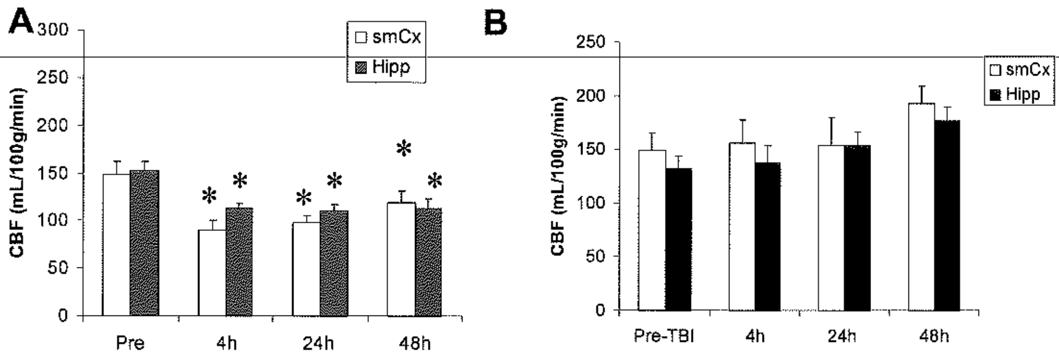


Figure 7. Combined effects of TBI and injection of BQ-123 on CBF.
A) CBF measurements using ASL-MRI 1 hour prior to (pre) TBI (using a modified Marmarou's acceleration impact model) and at 4, 24 and 48 hours post TBI (N=6 per group). As previously reported (Rafols et al., 2007a, Shen et al, 2007), TBI results in an approximately 35% decrease in CBF (hypoperfusion phase), which is sustained up to 48 hours post TBI. **B)** CBF measurements using ASL-MRI 1 hour prior to TBI, and at 4, 24, and 48 hours post TBI with bilateral ICV injection of BQ-123 (10 µg in 10 µl vehicle) given 30 min post TBI (N=8 per group). BQ-123 was able to ameliorate the hypoperfusion seen following TBI (as indicated by no significant differences in CBF).

Effects of ICV injection of BQ-123 at 30 min following TBI on performance in a radial arm maze.

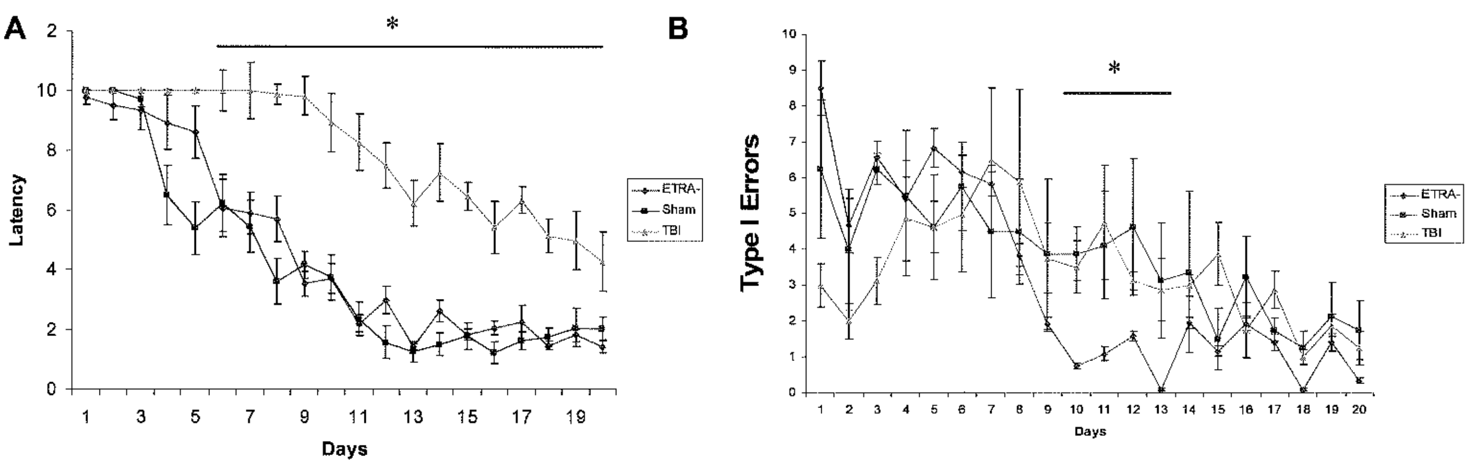


Figure 8. Combined effects of TBI and ICV injection of BQ-123 at 30 min post TBI on performance on a radial arm maze. Three groups of animals were used (N=12 per group). One received a sham operation only (pink), a second group received TBI (green) and the third group received TBI followed 30 min later with an ICV injection of BQ-123 (blue). Following treatment, animals were placed in a radial arm maze and latency (the time it took to retrieve all four pellets in **A**), type I errors (going down a non-baited arm in **B**) and type II errors (returning to a baited arm after food was already taken; data not shown) were recorded for 20 days straight during the dark (i.e., most active) cycle. BQ-123 was able to improve performance to nearly control (sham) levels and was able to (on days 10-13) improve the amount of Type I errors made. For more detail on how testing was conducted, see General Methods. * =p<0.05 compared to Sham using ANOVA with LSD post hoc.

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AIM 4. To test the hypothesis that ICV injection of the ETrB selective antagonist BQ-788 shortly after TBI will have little to no effect on hypoperfusion and will not improve cognitive outcome following TBI.

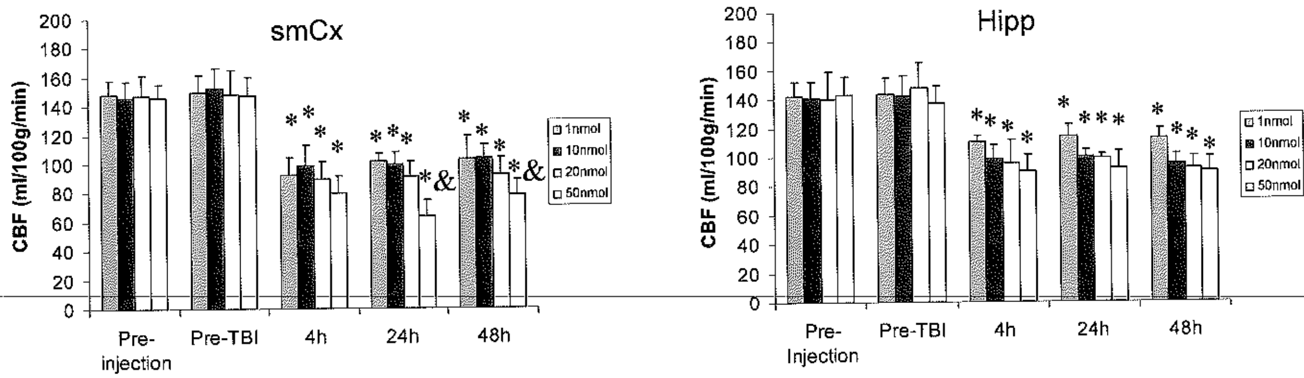

Figure 9. The effect of BQ-788 injection on TBI-induced hypoperfusion. Various doses of BQ-788 were administered 30 min post TBI and CBF was measured using ASL-MRI. Results show that no dose was effective in blocking TBI-induced hypoperfusion *and that the highest dose, 50nmol, consistently led to a further decrease.* N=5 per group. *= P<0.05 as compared with pre-TBI and &= P<0.05 as compared with 1nmol dose.

Effects of ICV injection of BQ-788 at 30 min following TBI on performance in a radial arm maze

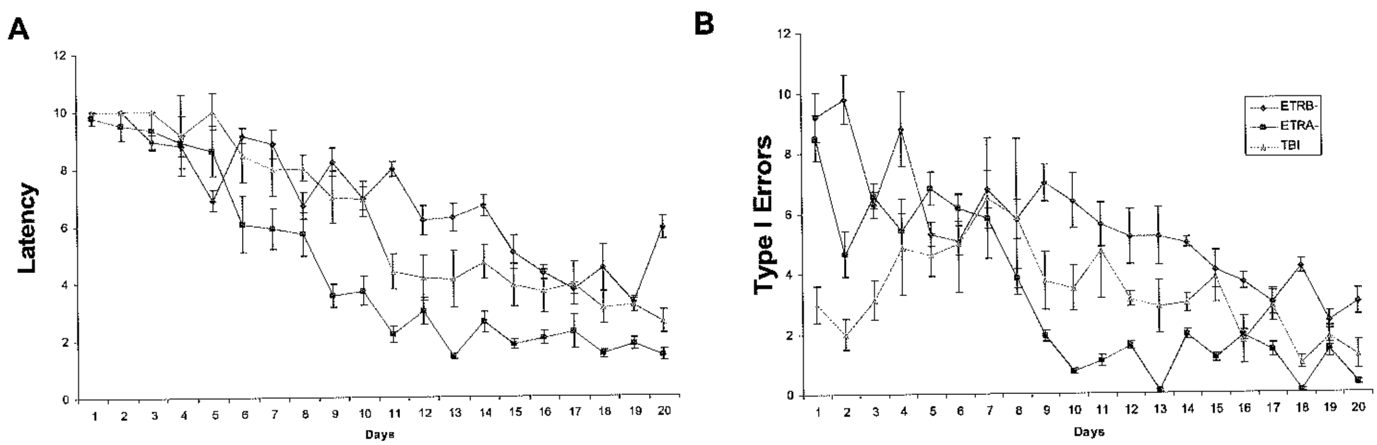

Figure 10. Comparison of the combined effects of TBI and ICV injection of BQ-123 or BQ-788 at 30 min post TBI on performance on a radial arm maze. Three groups of animals were used (N=6 per group). One received TBI (green) one received TBI followed 30 min later with an ICV injection of 40 µg BQ-123 (pink; same group as iFig 5 which is presented in blue) and one received TBI followed 30 min later with an ICV injection of 50 ng BQ-788. Following treatment, animals were placed in a radial arm maze and latency (the time it took to retrieve all four pellets in **A**), type I errors (going down an unbaited arm in **B**) and type II errors (returning to a baited arm after food was already taken; data not shown) were recorded for 20 days straight during the dark (i.e., most active) cycle. While BQ-123 results in improved performance, BQ-788 resulted in increased latency and an increase in Type I errors following TBI. For more detail on how testing was conducted, see General Methods.

SPECIFIC AIM 5 tests the hypothesis that improved CBF after TBI is mediated by ETrA antagonist-dependent upregulation of ETrB, thus allowing for vasodilation.

In this AIM we will test a potential mechanism by which ETrA antagonists may be exerting their effects on CBF following TBI. In order to develop this AIM we have conducted two preliminary experiments. In the first, we measured ETrA and B levels following either BQ-123 (ETrA antagonist) or BQ-788 (ETrB antagonist) administration in order to determine whether shifts in receptor expression could contribute the effect of antagonist injections. We determined that while BQ-788 had little to no effect on either receptor, BQ-123 caused a significant increase in ETrB levels at 24 and 48 hours post TBI. Therefore, we tested whether an injection of BQ-788 at 12 hours post TBI could ameliorate the improved *CBF and* behavioral outcome seen with BQ-123 administration.

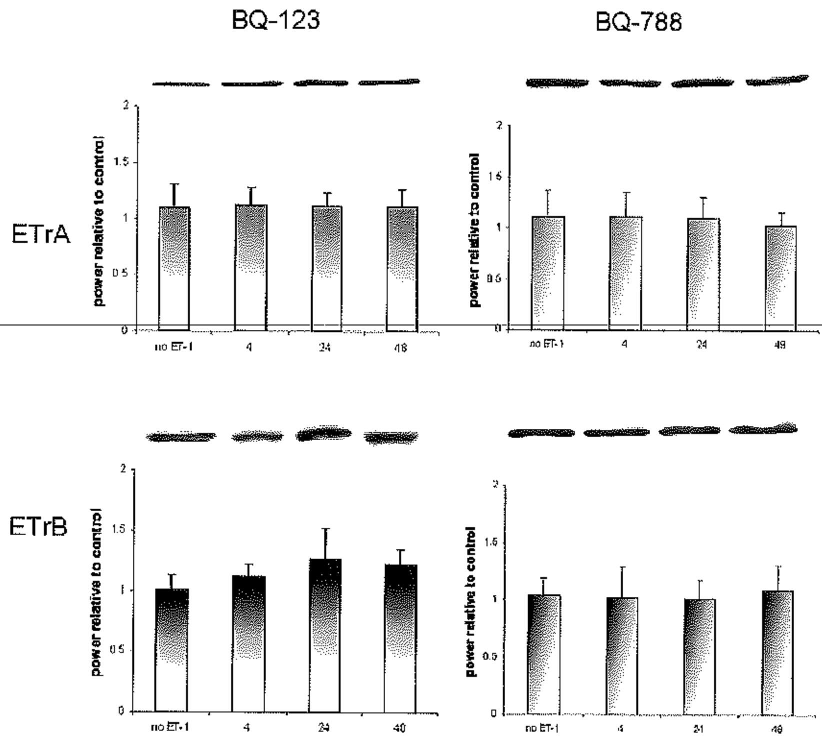

Figure 11. Effect of ETrA and B antagonists on ETrA/B expression after TBI. Here we injected either 40ng BQ-123 or 50ng BQ-788 30 min after TBI and measured ETrA/B protein levels at 4, 24 or 48hours post TBI (n=3 per group). Results indicate that BQ-788 had no effect on either ETrA or B protein expression. However, while BQ-123 had little effect on ETrA expression, it significantly increased expression of ETrB. These results suggest that ETrA antagonists may be effective in ameliorating TBI-induced decreases in CBF by upregulating the receptor responsible for vasodilation (i.e., ETrB).

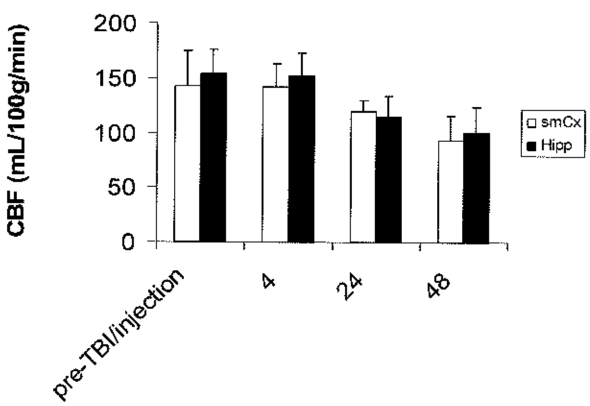

Figure 12. Effect of ETrB antagonist given at 11.5 hours post ETrA injection (given at 30 minutes post TBI). *Animals (n=3) were given IV injection of BQ-123 30 min following TBI. At 4 hours post TBI, CBF did not change, showing, once again, that ETrA blockade can ameliorate hypoperfusion. However, when BQ-788 (ETrB antagonist) was given at 11.5 hours (12 hours post TBI), blood flow at 24 and 48 hours post TBI was decreased from 4 hours, suggesting that ETrB blockade at later time points can reverse the effects of ETrA antagonists on TBI-mediated hypoperfusion.*

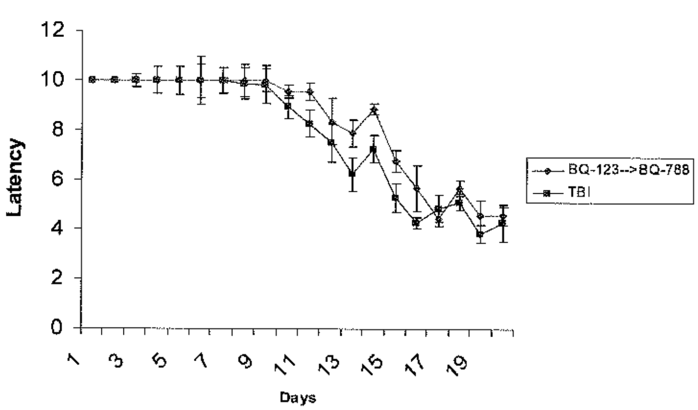

Figure 13. Effect of BQ-788 administered at 12 hours following BQ-123 on cognitive behavior. Recall from figure 8 that BQ-123 given after TBI significantly improved cognitive behavioral outcome as compared to vehicle injection. We speculate the therapeutic effect may be due to its ability to increase ETrB expression (figure 11), causing an increase in CBF to the brain after TBI, ultimately leading to increased neuronal activity and improved cognition. To test this, we administered BQ-788 at 12 hours (just prior to the observed increase in ETrB expression) following TBI and BQ-123 administration. Results indicate that by blocking ETrB, we ameliorated the observed improvement in behavioral outcome seen with only BQ-123. This suggests that the functional consequences of ETrA antagonism are mediated through increased expression of ETrB.

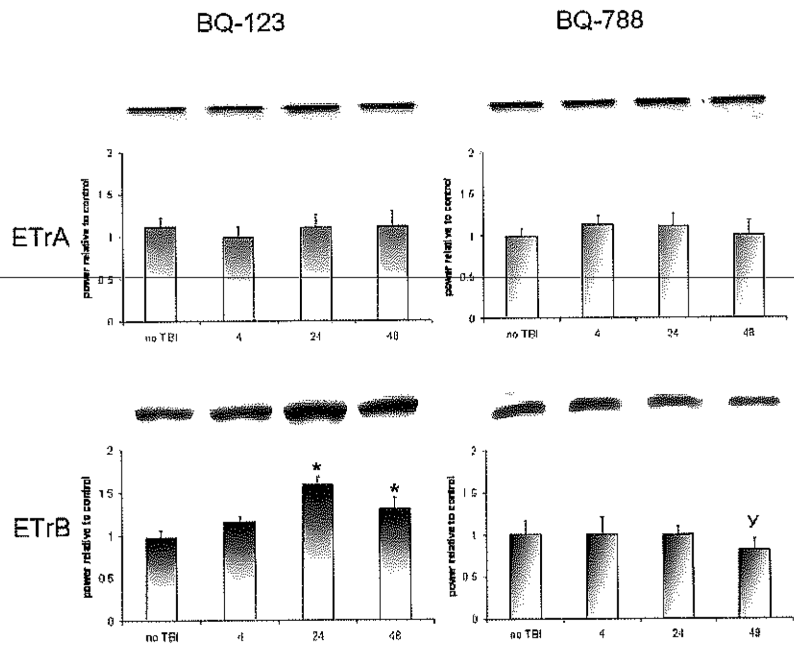

Figure 14. Effect of ETrA and B antagonists on ETrA/B expression afterET-1 injection. *In an effort to determine whether the receptor shifts seen after antagonist treatment following TBI are due to the effects of hypoperfusion, alone, or whether they result from TBI+hypoperfusion we injected either 40ng BQ-123 or 50ng BQ-788 30 min after ET-1 injection and measured ETrA/B protein levels at 4, 24 or 48hours post ET-1 injection (n=3 per group). Results indicate that BQ-788 had no effect on either ETrA or B protein expression. Furthermore, BQ-123 had little effect on ETrA or ETrB expression. This contrasts with the data seen in Figure 11 following TBI in which ETrB was significantly upregulated following BQ-123 treatment following TBI. These results suggest that TBI causes change in the mechanism by which ETrA antagonists act, causing an upregulation of ETrB receptors.*

D. RESEARCH DESIGN AND METHODS

SPECIFIC AIM 1 tests the hypothesis that alterations in rat cerebral perfusion, microvessel morphology, and endothelin (ET)-1 receptors (rA and rB) occurring after TBI are caused by ET-1 upregulation and that these changes contribute to histopathology and impaired spatial learning following injury.

Rationale. During the previous funding period we have determined that TBI results in: 1) increased vasoreactivity of reacting cerebral microvessels (Rafols et al., 2007a), 2) chronic hypoperfusion of brain starting at 4 hours post injury and continuing for up to 48 hours (Petrov and Rafols, 2002; Haacke et al., 2007), 3) significant upregulation of ET-1 receptors (A and B) in neurons and vascular cell types but not in glia (Kallakuri et al., 2007; Kreipke et al., in press), and 4) spatial memory cognitive deficits temporally associated with the persistent hypoperfusion (Kreipke et al., 2007c). All these observations temporally coincided with upregulation of ET-1 (protein and mRNA) in sensorimotor cortex (smCx) and hippocampus (Hipp) (Petrov et al., 2004). Since the first submission, we have now shown that ET-1 is upregulated in smCx and Hipp as early as 4 hours post TBI and persists until 24 hours, at which time it begins to normalize. This increase is correlated with changes in decreased CBF, microvessel wall conformational changes, ETrA/B expression, and poorer cognitive outcome. However, it is not yet known whether a rise in ET-1 brain levels in the normal (i.e., non-traumatized) rat alone might exert the aforementioned effects. Therefore, in this AIM, we seek to determine in naïve rats whether a predetermined dose of ET-1 delivered to brain which reduces cerebral blood flow (CBF) by an amount comparable to that seen after TBI, causes similar alterations in microvascular structure, histopathology and spatial memory as those reported in rats after TBI. Specifically, the following experiments in normal rats are designed to test whether an intracerebroventricularly (ICV)-delivered, predetermined ET-1 dose which causes a 35% decrease in the CBF (as seen in our TBI model) causes alterations in microvascular smooth muscle (SM) and luminal size, enhanced expression of ET-1 receptors (A and B) in selective neural types, and cognitive deficits similar to those observed after trauma. In doing so, we seek to determine whether ET-1 upregulation is sufficient to cause the observed sequellae of pathological events following TBI. To further determine whether ET-1 causes the specific changes mentioned, we will subject a well characterized ET-1 heterozygous knock-out mouse strain to TBI using an adaptation of Marmarou's model first described by Shahomi and colleagues (Chen et al., 1997). Overall, we hypothesize that ET-1 injections will cause similar pathologies seen following TBI while these observations will not occur in ET-1 knockout mice.

Experiment 1.1a: Determine the dose of ET-1 that results in an approximately 35% decrease in CBF, the percent change observed following TBI.

Here we have determined the amount of ET-1 increase in both Hipp and smCx that correlates with a 35% reduction in CBF following TBI (see preliminary data). We will inject various doses of ET-1 based on these levels directly into the brain (N=6) using guide cannulae directed at the lateral ventricles until CBF is reduced by approximately 35% of baseline value. By ASL-MRI we have previously published that TBI results in decreased CBF in both the smCx and Hipp, structures that are directly adjacent to the ventricles, and hence diffusion of ET-1 to both these areas is likely to occur after ICV injection. For confirmation, ET-1 will be tagged with FITC for immunofluorescent detection of the peptide. Two hours following injection (to allow for drug effect) we will measure CBF using ASL-MRI. These results will be compared with vehicle injected animals (N=6). Statistical significance of CBF among groups will be established by the Student's T test.

Expected Results: Based on preliminary results, we predict that, compared to injection of vehicle alone, a 0.2ng dose of ET-1 delivered in 10µl total volume will yield a 35% reduction in CBF in smCx and Hipp as determined by ASL-MRI.

Possible pitfalls, alternative approaches: Given that ET-1 upregulation persists for 24 hours, it is possible that one ICV injection of ET-1 will not be sufficient to mimic the changes seen in TBI. Therefore we are prepared to incorporate a multi-injection approach which can easily be achieved through the cannulae to mimic the time course seen after TBI. However, based on the preliminary data, we predict that one injection will be sufficient to cause significant changes. It is possible that ICV injection will not reproduce the diffuse distribution of ET-1 upregulation following TBI. However, since ASL-MRI allows for a multi-regional analysis, we will be able to determine ET-1 effects in various regions simultaneously, including smCx and Hipp.

Experiment 1.1b: Determine whether the above ET-1 effect on CBF can be blocked by ICV or IV administration of BQ-123, a well-characterized selective ETrA antagonist, or Clazosentan, a relatively new drug which has, as aforementioned, scarcely been used in the brain.

CBF will be determined 1 hour prior to ET-1 injection (optimal dose to be determined in experiment **1.1a**). One hour after ET-1 injection, we will inject various doses of BQ-123 or Clazosentan via ICV or IV injection (1, 5, 10ng, N=8

per group, ICV; 10, 20, 50mg-kg, N=8 per group, IV). We will then measure CBF using ASL-MRI. Statistical significance and drug effect will be determined using ANOVA with LSD post hoc, comparing ET-1 injection followed by vehicle, ET-1 injection followed by BQ-123, vehicle injection following by vehicle, and vehicle injection followed by BQ-123.

Expected Results: Based on previously reported ETrA effects on vasoconstriction and our provided preliminary results, we predict that administration of BQ-123 prior to ET-1 delivery will block the latter's ability to significantly decrease the CBF, thus resulting in improved CBF. Also based on preliminary data, we predict Clazosentan will have similar effects.

Possible pitfalls, alternative approaches: Due to the potential presence of other vasoconstrictors in the brain, it is possible that the administered dose of BQ-123/Clazosentan will not be sufficient to entirely block the observed decreases in CBF. However, we do not anticipate this being a problem as ET-1 has not been shown to have effects on regulation of other vasoconstrictors. Further, since ETrA exists in other cells than just vascular components, it is possible that ICV v. IV injections will yield differential effects. While our preliminary data suggests that both are equally as effective, if differences do exist, we will be able to begin to understand the role of these receptors in non vascular subunits.

<u>Experiment 1.1c:</u> Determine whether the ET-1 effect on CBF can be blocked by BQ-788, a selective ETrB antagonist.

Here we will inject various doses of BQ-788 via ICV or IV injection (1, 5, 10ng, N=8 per group, ICV; 10, 20, 50mg-kg, N=8 per group, IV) 1 hour after ET-1 injection (optimal dose to be determined in experiment **1.1a**). 1 hour after BQ-788 injection we will measure CBF using ASL-MRI. Statistical significance and drug effect will be determined using ANOVA with LSD post hoc, comparing ET-1 injection followed by vehicle, ET-1 injection followed by BQ-788, vehicle injection followed by vehicle, and vehicle injection followed by BQ-788.

Expected Results: Based on previously reported ETrB effects on vasodilation and our provided preliminary results, we predict that the administered BQ-788 will have no effect or will increase ET-1's ability in further reducing CBF (at high doses only) both via enhanced vasoconstriction and/or attenuation of vasodilation.

Possible pitfalls, alternative approaches: As our preliminary data suggests, BQ-788 was not sufficient to decrease CBF beyond the decrease seen by ET-1 injection alone. Therefore, we may not be able to detect whether BQ-788 can exacerbate the ET-1-induced decrease in CBF. However, this finding will not diminish our overall ability to interpret the action of ET-1 in regulating CBF in the normal, non-injured brain.

<u>Experiment 1.1d:</u> Determine whether the ET-1 effect on CBF can be blocked by Bosentan, a mixed ETrA/B antagonist.

Here we will inject various doses of Bosentan via ICV or IV injection (1, 5, 10ng, N=8 per group, ICV; 10, 20, 50mg-kg, N=8 per group, IV) 1 hour after ET-1 injection (optimal dose to be determined in experiment **1.1a**). 1 hour after Bosentan injection we will measure CBF using ASL-MRI. Statistical significance and drug effect will be determined using ANOVA with LSD post hoc, comparing ET-1 injection followed by vehicle, ET-1 injection followed by Bosentan, vehicle injection following by vehicle, and vehicle injection followed by Bosentan.

Expected Results: Based on previously reported effects of Bosentan on ETrB and vasodilation and our provided preliminary results, we predict that the administered Bosentan will have no effect on ET-1's ability in further reducing CBF.

Possible pitfalls, alternative approaches: While the effects of Bosentan in the brain have not been elucidated, it is possible that the block of ETrA can override its effects on ETrB, and thus may act more like B-123. However, given the relatively poor outcome of Bosentan seen in the clinic, we predict that it will not be sufficient to ameliorate CBF changes following ET-1 injection.

<u>Experiment 1.2a:</u> Determine whether ET-1 injection into normal brain results in morphological alterations of microvascular smooth muscle (SM) and endothelium which may underlie enhanced vasoreactivity.

Here we will utilize brain tissue from animals that received an optimal dose of ET-1 resulting in 35% reduction in CBF from experiment **1.1**. Tissue will be perfused and processed for electron microscopy (EM). Qualitative analyses of SM in reacting microvessels (i.e., terminal and precapillary arterioles) will be conducted to detect evidence of enhanced SM contractility (e.g., increased ruffling and interdigitations of sarcolemma from adjacent SM cells). In addition measurements of luminal areas of microvessels in smCx and Hipp will be conducted directly from semi-thin (1.0um thick/toluidine blue stained) and ultrathin (EM) images using stereological methods (see General Methods). The resulting data from non-traumatized brain will be compared to that from TBI brains previously published (Rafols et al., 2007a).

Expected Results: We expect that ET-1 administration will result in enhanced SM contractility as well as a decrease in the average luminal area of microvessels similar to those observed following TBI.
Possible pitfalls, alternative approaches: As with any EM study, sampling errors due to the small area of analysis in ultrathin sections are possible. However, here we use both semi-thin (light) and ultrathin (EM) preparations in order to allow us to sample from a relative large number of blood vessel profiles in a given area, as well as for achieving accuracy in terms of magnification. We have a proven track record for performing such studies and have previously reported on measurements of luminal areas of microvessels after sampling from several areas in a given region of analysis.

Experiment 1.2b: Test the effect of an IV or ICV injection of BQ-123 or Clazosentan (doses determined above) on enhanced ET-1-induced vascular contractility. As in **1.2a**, qualitative and stereological quantitative analyses of SM and luminal sizes of reacting microvessels in smCx and Hipp will be carried out.
Expected Results: Because the documented (Povlishock et al., 1983), role of ETrA in vasoconstriction we predict that BQ-123 or Clazosentan administration after ET-1 injection will result both in decreased SM contractility and increased average luminal area of microvessels.
Possible pitfalls, alternative approaches: We are well aware that we will have to perform a dose response to optimize the efficacy of BQ-123 or Clazosentan. This is not deemed to be a problem, however, since we can alternatively monitor CBF as an indirect measure of vasoconstriction using ASL-MRI. Using this approach we do not need to increase the number of animals as multiple injections can be performed in the same animal.

Experiment 1.2c: Test the effect of a IV or ICV injection of BQ-788 (dose determined above) on enhanced ET-1-induced vascular contractility.
Expected Results: Due to ETrB's vasodilatory properties (Randall et al., 1989), we predict that BQ-788 administration prior to ET-1 injection will exacerbate the vasoconstriction effects of ET-1 by increasing both SM contractility and decreasing the average luminal area of reacting microvessels in smCx and Hipp. These effects will be shown by conducting the same qualitative and morphometric analyses as in **1.2a** and **1.2b**.
Possible pitfalls, alternative approaches: It is possible that other vasoactive substances (e.g., nitric oxide, angiotensin, purines) could mask the effects of BQ-788, thus showing that the observed enhanced vasoconstriction might not be mediated exclusively via ETrB blockage. However, based on our ASL-MRI preliminary data we have evidence of a trend towards increased vasoconstriction and, thus, do not foresee any caveats to this experiment.

Experiment 1.2d: Test the effect of an IV or ICV injection of Bosentan (dose determined above) on enhanced ET-1-induced vascular contractility.
Expected Results: We predict that administration of the mixed antagonist Bosentan will not change the ultrastructure of microvessels after TBI. This will be shown by conducting the same qualitative and morphometric analyses as in **1.2a** and **1.2b**.
Possible pitfalls, alternative approaches: As in Experiment **1.1d**, it is possible that the block of ETrA can override its effects on ETrB, and thus may act more like B-123. However, we predict, based on the lack of conclusive effects in the clinic, it will have little effect on ET-1 induced vasoconstriction.

Experiment 1.3: To test whether ET-1 injection in the normal, non-injured brain alters ETrA and ETrB expressions (protein and mRNA) and localization similar to those observed following TBI.

Following injection of an optimal dose of ET-1 as determined in experiment **1.1**, we will then utilize non-perfused smCx and Hipp tissue samples for Western analysis of ETrA and ETrB protein levels as well as RT-PCR analysis for the receptors mRNA levels. Given that both ETrA and ETrB have been shown to be expressed in non-vascular cell types (e.g., glia, neurons), in order to elucidate vascular versus non-vascular expression we will run PCR and Western analyses of smCx and Hipp tissue resections as well as on preparations that include only vascularization (see general Methods for protocol for extractions of vascular SM and endothelium. Additionally we will utilize immunocytochemistry and double-label immunofluorescent techniques to confirm the localization and compartmentalization of the receptors in discrete cell types (see General Methods for more detail). Statistical analysis will be accomplished using ANOVA with LSD post hoc.
Expected Results: We predict that, similarly to what it occurs after TBI, ET-1 injection will result in increased synthesis and expression of ETrA and ETrB in both smCx and Hipp. Our preliminary Western results in a limited number of animals show that ET-1 increases ETrA expression in both these brain regions.
Possible pitfalls, alternative approaches: It is possible that increases in ET-1 brain levels alone are not sufficient to cause an upregulation of the receptors. Alternatively TBI may cause additional changes that result in the observed increase in the receptors. We wish to point out that such findings would be significant in further understanding how TBI might lead to dysfunctional control of microvascular tone and, thus, negative results in this

AIM would provide valuable information, nonetheless. However, based on our preliminary results, we predict that increased presence of ET-1in brain will in itself alter ETrA and B expression.

Experiment 1.4: *To determine whether ET-1 injection-mediated hypoperfusion results in cell injury.*

Here we will utilize brain tissue taken from animals in Experiment 1.1, section for analysis of Hipp and smCx and measure the extent of FluoroJade labeling to determine whether ET-1 mediated hypoperfusion results in cell injury.
Expected Results: *We predict, based on preliminary data, that ET-1 injection will cause cell injury as evidenced by FluoroJade-positive cells within Hipp and smCx.*
Possible pitfalls, alternative approaches: *While our preliminary data suggests that we will have no problems in determining extent of cell injury, we may not be able to conclusively determine from this experiment whether cell injury is caused by ET-1 hypoperfusion or by another mechanism. However, AIM 2 is dedicated to resolving this issue (Please see AIM 2).*

Experiment 1.5a: To determine whether ET-1 injection results in cognitive deficits.

Here we will administer an ICV injection of ET-1 once daily for three days in order to mimic the sustained state of hypoperfusion seen following TBI. Following final injection we will commence cognitive behavioral testing as described in General Methods to determine whether ET-1 injection causes decreased performance on the radial arm maze. The results from this experiment will be compared to data obtained from animals that received ICV injection of vehicle (saline) only. Statistical significance will be determined by comparing latencies and number of errors across time using ANOVA with LSD post-hoc test.
Expected Results: We predict that ICV injections of ET-1 for three consecutive days will result in decreased performance on the radial arm maze.
Possible pitfalls, alternative approaches: While we are ultimately attempting to correlate the already published deficits in cognitive behavior following TBI with those seen following ET-1 injection, it is possible that ET-1 injection will not result in the same profile of cognitive deficit as TBI. In addition to CBF alterations TBI also brings about diffuse axonal injury which is likely to contribute to the observed behavioral deficits. Because of this, an added benefit of the experiment will be finding out the relative contribution of decreased CBF to overall cognitive performance in normal (i.e., non-traumatized) animals. In addition we will use the Morris water maze as an alternate method to the radial arm maze to assess spatial learning and cognitive outcome.

Experiment 1.5b: To test the effect of ICV or IV injection of BQ-123 or Clazosentan (doses determined as above) on ET-1 effects on cognitive behavior.

Here we will test as in **1.5a**, but with an ICV or IV injection of BQ-123 or Clazosentan 1 hour post ET-1 injection. These results will be compared with animals receiving vehicle injection after ET-1. Statistical significance and drug effect will be determined by comparing latencies and number of errors across time using ANOVA with LSD post-hoc test.
Expected Results: As in experiment **1.2b**, we predict ETrA blockage to ameliorate the effects of ET-1 on cognitive behavior. Such improvement is deemed to be the result of an improved CBF.
Possible pitfalls, alternative approaches: If ET-1 injection in the non-injured brain did not result in cognitive deficits, then we may acknowledge that this experiment is unlikely to yield interpretable results. However as the preliminary results suggest, ET-1 injection causes impaired cognition although additional data needs to be generated to determine the contribution of ETrA and CBF to spatial learning.

Experiment 1.5c: To test the effect of ICV or IV injection of BQ-788 on ET-1 effects on cognitive behavior.

Here we will proceed as in **1.5a**, but with an ICV or IV injection of BQ-788 1 hour after Et-1 injection. These results will be compared with animals receiving vehicle injection prior to ET-1. Statistical significance and drug effect will be determined by comparing latencies and number of errors across time using ANOVA with LSD post-hoc test.
Expected Results: As in experiment **1.2c**, we predict that ETrB blockage by Iv injection of BQ-788 after ET-1 delivery will result in poorer performance in the radial arm maze compared to vehicle injected controls. This worsening of the cognitive performance is probably due to both diminished vasodilation and enhanced vasoconstriction which further reduces the CBF.
Possible pitfalls, alternative approaches: As in experiment **1.2c**, other vasodilatory substances such as NO may contribute to vasodilation and, thus, we may not see appreciable differences between vehicle and BQ-788 injected animals with respect to performance on the radial arm maze. However, our preliminary data, albeit conducted on only 6 animals, shows a trend towards decreased spatial learning and, hence, we do not predict any major caveats

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to this experiment. Additionally, we will use the Morris water maze as an alternate method to the radial arm maze to assess spatial learning and cognitive outcome.

Experiment 1.5d: To test the effect of ICV or IV injection of Bosentan on ET-1 effects on cognitive behavior.

Here we will proceed as in **1.5a**, but with an ICV or IV injection of Bosentan 1 hour after Et-1 injection. These results will be compared with animals receiving vehicle injection prior to ET-1. Statistical significance and drug effect will be determined by comparing latencies and number of errors across time using ANOVA with LSD post-hoc test.

Expected Results: As in experiment **1.2d**, we predict that Bosentan injection after ET-1 delivery will result in poor performance in the radial arm maze compared to normal animals. This worsening of the cognitive performance is probably due to both diminished vasodilation and enhanced vasoconstriction which further reduces the CBF.

Possible pitfalls, alternative approaches: As in experiment **1.2d**, it is possible that the block of ETrA can override its effects on ETrB, and thus may act more like B-123. However, we predict, based on the lack of conclusive effects in the clinic, it will have little effect on ET-1 induced vasoconstriction.

Experiment 1.6: To further test the causal relationship between ET-1 with TBI-induced changes in CBF, vasoreactivity, and cognitive outcome (Experiments 1.1-1.4), we have added a new experiment since the first submission. This experiment utilizes a well characterized heterozygous ET-1 knock-out mouse (ET-1$^{+/-}$) (see Kuwaki et al., 1997) which will be subjected to diffuse TBI by using a weight acceleration impact mouse model modified by Shahomi and colleagues (Chen et al., 1997) after Marmarou's model in rats. As explained in the new preliminary data the heterozygous knockout is viable to adulthood and is physiologically normal yet expresses only 40% of normal, wild-type ET-1 levels. These results will be compared with the wild type, ET-1$^{+/+}$, to test whether diminished expression of ET-1 will decrease the amount and extent of hypoperfusion following TBI. In doing so, we will indirectly confirm whether ET-1 causes enhanced vasoreactivity following TBI.

Here we will subject ET-1$^{+/-}$ mice to TBI and will measure CBF, ultrastructural changes in microvessels, ETrA/B spatial changes, and cognitive performance following TBI.

Expected Results: As we hypothesize that ET-1 causes the above outlined changes after TBI, by decreasing ET-1 levels by 60% we predict that we will observe little to no change in CBF following TBI, no change in ultrastructure of microvessels, no changes in the spatial localization of ETrA/B and no major deficits in cognitive performance following TBI.

Possible pitfalls, alternative approaches: It is possible that diminished ET-1 may cause upregulation of other vasoactive substances which could confound our results. However, as first characterized by Kumada and colleagues (Kuwaki et al., 1997), no profound changes in either ET-1 receptors or in peripheral blood pressure have been observed in these animals. Therefore we predict that other biological systems will be intact and thus will not confound our interpretation of the results.

SPECIFIC AIM 2 tests the hypothesis that TBI-mediated hypoperfusion exacerbates cell injury and contributes to behavioral deficits following head trauma.

Rationale. *Thus far our overall hypothesis assumes that the primary result of ET-1 and ETrs changes after TBI is hypoperfusion which, in turn, leads to cell damage and poorer behavioral outcome. However, it is noted in the background and significance section that ETrs are not only expressed in vascular compartments, but also in a host of other cell types (Kallakuri et al., 2007; in press). Further, as detailed in the background and significance section it has been suggested that changes in ETrs can have direct effect on the viability of neurons. Therefore, this AIM will utilize a technique to decrease CBF downstream of ETrs, thus testing whether TBI-mediated hypoperfusion, itself, is a primary factor in poor outcome. In this set of experiments we will, independently of ETr antagonism, block the phosphorylation of Cp, a contractile protein, following TBI which, in turn, prevents the ability of a vessel to contract. In this way, as previously shown (Kreipke and Rafols, 2009), we can successfully block hypoperfusion following TBI. We will then test the same outcome measures as in AIM 1, i.e., extent of cell injury using FluoroJade and behavioral outcome to see whether upregulation of ETrs mediates a change in CBF which exacerbates TBI-induced cell injury and behavioral deficits, or whether there is an ETr mechanism involved which is independent of CBF.*

Experiment 2.1: *Here we will test the effects of blocking TBI-induced hypoperfusion, independent of a direct ETr mechanism, by blocking Cp phosphorylation. We will inject anti-Cp via ICV injection 1 hour before TBI which, as previously published (Kreipke and Rafols, 2009) is sufficient to block TBI-mediated hypoperfusion . TBI will then be induced and at 4, 24 and 48 hours post TBI brains will be harvested, sectioned, and stained for FluoroJade labeling. Results will be compared with those of vehicle injected TBI-animals and with the results of Experiment 1.4. In this*

way, we can both determine how blocking hypoperfusion impacts on the extent of cell injury following TBI and what the contribution of hypoperfusion is to cell injury, in the absence of potential vascular-independent ET-1 effects.
Expected Results: *We predict, based on preliminary data, that blocking TBI-mediated hypoperfusion with anti-Cp will reduce the extent of cell injury.*
Possible pitfalls, alternative approaches: *One potentially minor difficulty may be in quantifying the extent of FluoroJade labeling between groups. However as we have a combined experience of over 30 years in conducting such an analysis, we do not predict any major caveats to this experiment.*

Experiment 2.2: *Here we will administer anti-Cp as in Experiment **2.1** and, 24 hours post TBI we will measure behavioral outcome as in Experiment **1.5a**. Results will be compared with animals receiving vehicle injection and with those in Experiment **1.5a** to determine both how blocking hypoperfusion impacts on behavioral outcome following TBI and what the contribution of hypoperfusion is to cognitive deficits, in the absence of potential vascular-independent ET-1 effects.*
Expected Results: *We predict, based on preliminary data, that blocking TBI-mediated hypoperfusion with anti-Cp will improve behavioral outcome.*
Possible pitfalls, alternative approaches: *We do not predict any major caveats to this experiment.*

SPECIFIC AIM 3 tests the hypothesis that ICV or IV injections of the ETrA antagonist BQ-123 or a potentially clinically relevant drug, Clazosentan, directly following TBI will ameliorate hypoperfusion and improve cognitive outcome.

Rationale. Previously we have shown by immunocytochemistry (ICC) that TBI results in a significant increase in ETrA expression in neurons and cells of the microvascular wall (Kallakuri et al., 2007; Kreipke et al., in press). These increases were temporally correlated with enhanced expression and intracellular trafficking of key contractile proteins and signaling in SM (Kreipke et al., 2006, 2007a, b). Mechanistically the known downstream effect of ETrA activation is vasoconstriction. Given that these increases in ETrA expression and signaling temporally coincide with the state of sustained hypoperfusion observed following TBI (Rafols et al., 2007), ETrA activation likely causes increased vascular tone and, hence, blocking this receptor would lead to shortening the duration and extent of the hypoperfusion following injury. Therefore, here we seek to determine the effect of ICV injection of BQ-123, a well-characterized selective antagonist of ETrA following TBI on both CBF and cognitive outcome. To strengthen the clinical correlation of these experiments we will compare this data with IV injections of BQ-123. Furthermore, we will test both ICV and IV injection of a novel ETrA antagonist, Clazosentan. We will first perform a dose response of BQ-123 and Clazosentan to determine the optimal concentration needed to reverse the TBI-induced decrease in CBF. Next we will use the optimal dose at different times following injury to determine the "window of opportunity" for intervention. Finally, we will test the optimal dose given at the time determined in the second experiment and test its effects on cognitive outcome using the radial arm maze test. All results will include a comparison of sham operated versus TBI, vehicle injected versus drug injected animals. It should be noted that while ET-1 receptor antagonism to modify CBF after TBI is an ongoing objective of the presently funded grant, the present Aim differs significantly from that of the current grant in two ways. First injections here will be delivered post-TBI rather than before injury as in the current proposal. Second, the proposed outcomes here will be in tandem ASL-MRI blood flow and cognitive behavior assessments using technologies which were not available at the time of the current grant application. We are encouraged that using these improved methods positions us closer to design an effective therapeutic strategy to improve CBF after TBI.

Experiment 3.1: Determine the effect of ICV and IV injection of various doses of BQ-123 or Clazosentan on CBF following TBI.

Here we will first measure CBF using ASL-MRI in 120 animals. Following this, 8 animals will be given sham operation (group 1) and TBI will be administered to 112 animals to be used in the following groups (N=8 animals per group): (2) TBI only, (3) vehicle, (4-5) 10ng (ICV), (6-7) 20ng (ICV), (8-9) 40ng(ICV) BQ-123 or Clazosentan and (10-11) 10mg/kg (IV), (12-13) 20mg/kg μg (IV), (14-15) 50mg/kg (IV) BQ-123 or Clazosentan. In the TBI only group, CBF will be measured 4 hours after surgery. In all other groups BQ-123/Clasoentan will be administered ICV or IV 30 min after TBI. CBF will be measured 4 hours after surgery. All animals will have CBF measurements at 24 and 48 hours. All groups will be compared using ANOVA with LSD post-hoc test to determine the effects of BQ-123 on CBF following TBI.
Expected Results: Based on preliminary data, we expect both ICV and IV injections of BQ-123 to block the ETrA-mediated decrease in CBF following TBI in a dose-dependent manner. Based on preliminary data and due to its high efficacy to ETrA, we predict Clazosentan to have similar results.

Possible pitfalls, alternative approaches: A caveat to this experiment is that since Clazosentan has scarcely been used in the brain, its effects are potentially unknown. However, since it has high efficacy to ETrA we do not foresee any problems.

Experiment 3.2: Determine the effect of ICV or IV-administered BQ-123/Clazosentan at various latter time points post-TBI on CBF.

In this experiment we will use the optimal dose of BQ-123 or Clazosentan as determined in experiment **3.1** at various time points (1 hr, 1.5 hr and 2 hr) following injury. The obtained effects on CBF will be compared to those from experiment **3.1** to determine the optimal time for drug intervention. Statistical significance will be determined by comparing drug to no drug using Student's T-test.
Expected Results: Since the observed peak hypoperfusion does not appear until approximately 4 hours post-TBI, we predict that BQ-123/Clazosentan injection up to 2 hours post impact will result in significantly blunting the extent and duration of this phenomenon.
Possible pitfalls, alternative approaches: We do not predict any caveats here.

Experiment 3.3: Determine the effect of BQ-123 or Clazosentan injection following injury on cognitive behavioral outcome.

Here we will administer the optimal dose of BQ-123 or Clazosentan (as determined in experiment **3.1**) at the optimal time (as determined in experiment **3.2**) and test its effects on cognitive outcome following TBI. Results from drug-injected animals will be compared with vehicle injections to determine drug effect. Significance will be determined by comparing latency and number of errors across all time points using ANOVA with LSD post hoc test.
Expected Results: Based on preliminary results, we predict that BQ-123 and Clazosentan will restore performance on the radial arm maze to near baseline levels (sham operated controls).
Possible pitfalls, alternative approaches: As noted in the introduction, hypoperfusion is not the only pathology of TBI. TBI also results in brain edema and diffuse axonal injury which could also affect cognitive outcome. Therefore we may not see a complete recovery in function. However, we would like to point out that the goal of this experiment is to see if BQ-123 or Clazosentan has an effect on cognitive behavioral outcome and, therefore, we are not suggesting that BQ-123 or Clazosentan will completely reverse all deficits, merely that it will help to improve outcome faster than would occur with only spontaneous recovery. Furthermore, we have begun investigations as to whether improving blood flow can improve neuronal outcome or decrease the extent of DAI. While this would add substantial work to this proposal, we feel that this investigation would decrease the focus of this work on cerebrovascular effects after TBI and thus is beyond the scope of this proposal.

SPECIFIC AIM 4 tests the hypothesis that ICV or IV injection of an ETrB antagonist (BQ-788) or a mixed ETrA/B antagonist, Bosentan directly following TBI will have no effect on TBI-induced hypoperfusion and will not improve cognitive outcome following TBI.

Rationale. As stated in the Background and Significance section, the role of ETrB in controlling microvascular tone is somewhat controversial. It has been reported to act as both a vasodilator (Randall et al., 1989; Hasunuma et al., 1990; Fukuroda et al., 1994; Ivy et al., 1994; Sato et al., 1995) and a vasoconstrictor (Clozel et al., 1992; Harrison et al., 1992; Moreland et al., 1992; Teerlink et al., 1994). While it has been suggested that, due to this disparity, two subtypes, ETrB1 and ETrB2 of ETrB exist, only subtypes ETrA and B have been cloned (Cazaboun and Courand, 1998; Nakagomi et al., 2000; Ho et al., 2001). In endothelium, ETrB is thought to mediate vasodilation through ET-1 clearing and nitric oxide (NO) release (reviewed in Pollack and Schneider, 2006). The activity of ETrB may be related to its localization. In endothelium, ETrB is thought to mediate vasodilation through ET-1 clearing and nitric oxide (NO) release (reviewed in Pollack and Schneider, 2006). However, in one study it was shown that ETrB exerts a vasoconstrictive force within pulmonary smooth muscle cells (Perreault and Baribeau, 1995). Another study showed that an initial dose of 1μmol of BQ-3020 applied directly to pial arterioles resulted in vasodilation while subsequent doses in the same vessel given 10 min apart resulted in no effect followed by vasoconstriction (Touzani et al., 1997). These disparities, coupled with the fact that mixed ETrA and B antagonists have been shown to have little effect in the clinical setting, we hypothesize that in brain, ETrB facilitates vasodilation, antagonism of this receptor thus resulting in enhanced vasoconstriction. We have previously presented work showing that ETrB antagonism prior to TBI worsens cognitive outcome. However, a more detailed analysis of its effects following TBI is needed. Therefore, in this AIM we will directly test the effects of ETrB antagonism following TBI using the selective ETrB antagonist, BQ-788. We will also use Bosentan, a mixed ETrA/B antagonist, which has been used in the clinical setting with mixed results (see Background and Significance).

Experiment 4.1: Determine the effect of ICV or IV injection of various doses of BQ-788 or Bosentan on CBF following TBI.

Here we will first measure CBF using ASL-MRI in 120 animals. Following this, 8 animals will be given sham operation (group 1) and TBI will be administered to 112 animals to be used in the following groups (N=8 animals per group): (2) TBI only, (3) vehicle, (4-5) 10ng (ICV), (6-7) 20ng (ICV), (8-9) 40ng (ICV) BQ-788 or Bosentan and (10-11) 10mg/kg (IV), (12-13) 20mg/kg µg (IV), (14-15) 50mg/kg (IV) BQ-788 or Bosentan. In the TBI only group, CBF will be measured 4 hours after surgery. In all other groups BQ-788 will be administered ICV or IV 30 min after TBI. CBF will be measured 4 hours after surgery. All animals will have CBF measurements at 24 and 48 hours. All groups will be compared using ANOVA with LSD post-hoc test to determine the effects of BQ-788 on CBF following TBI.
Expected Results: We expect both BQ-788 and Bosentan to have no effect or at higher doses even enhance the decrease in CBF following TBI in a dose-dependent manner.
Possible pitfalls, alternative approaches: It is possible that an approximately 35% decrease in CBF is at the threshold of how far CBF can drop and thus further decreases may be difficult to detect. However, based on our preliminary data, we predict that with the proper power analysis we can detect further increases in blood flow.

Experiment 4.2: Determine the effect of ICV or IV injection of BQ-788 or Bosentan given at various time points following TBI on CBF.

In this experiment we will use the optimal dose of BQ-788 or Bosentan as determined in experiment **4.1** at various time points (1 hr, 1.5 hr and 2 hr) following injury. The obtained results will be compared to those from experiment **4.1** to determine the optimal time for drug intervention. Statistical significance will be determined by comparing drug to no drug using Student's T-test.
Expected Results: We predict that BQ-788 or Bosentan injection up to 2 hours post impact will result in no change in CBF except at the highest doses where, based on preliminary data, a slight exacerbation of hypoperfusion was detected.
Possible pitfalls, alternative approaches: We do not predict any major caveats to this experiment.

Experiment 4.3: Determine the effect of BQ-788 or Bosentan injection following injury on cognitive behavioral outcome.

Here we will administer the optimal dose of BQ-788 or Bosentan (as determined in experiment **4.1**) at the optimal time (as determined in experiment **4.2**) and test its effects following TBI. Results from drug-injected animals will be compared with vehicle injections to determine drug effect. Significance will be determined by comparing latency and number of errors across all time points using ANOVA with LSD post hoc test.
Expected Results: Based on preliminary data, we predict that both BQ-788 and Bosentan will have no effect or even worsen cognitive outcome following TBI.
Possible pitfalls, alternative approaches: It may be difficult to determine a worsening of cognitive outcome following TBI due to the already compromised performance on the radial arm maze and the fact that we stop the test at 10 minutes. However, the overall goal is to ascertain whether ETrB antagonism has any effect and therefore, we will still be able to tell over time whether ETrB antagonism impairs cognition.

SPECIFIC AIM 5 tests the hypothesis that improved CBF after TBI is mediated by ETrA antagonist-dependent upregulation of ETrB, thus allowing for vasodilation.

Rationale. The previous three AIMs are designed to test whether upregulation of ET-1 causes decreased CBF following TBI (AIM 1) and whether ETrA antagonists can prove effective in ameliorating these decreases in CBF and behavioral deficits that result from the state of chronic hypoxia (AIMS 2, 3). While the preliminary data appears hopeful that these antagonists could be useful in the clinical setting, we have not addressed the mechanism by which these drugs are working. Recall from the Background and Significance that reports suggest that ETrA mediates primarily vasoconstriction while ETrB is vasodilatory. Furthermore, the clinical efficacy of mixed antagonists (i.e., antagonize both ETrA and B) has seen limited success. Therefore we have designed a series of experiments to investigate the functional relationship between ETrA/B in the presence of the receptors' antagonists. In doing so, we will contribute to an understanding of how these receptors may contribute, overall, to stabilizing vasoreactivity following TBI and how to most effectively implement therapies aimed at either receptor. The following experiments have been designed to test the hypothesis that ETrA antagonists upregulate ETrB, allowing for vasodilation and improved CBF following TBI. *As a final confirmation of whether these changes are the result of hypoperfusion, alone, or TBI's effects in the presence of hypoperfusion, we will repeat experiments in this AIM using the same ET-1 injections as in AIM 1 in the absence of TBI.*

Experiment 5.1: Determine the effect of BQ-123 or Clazosentan on levels of ETrA/B mRNA and protein after TBI.

Here we will induce TBI. 30 minutes following TBI we will inject either BQ-123 or Clazosentan IV based on effective doses seen above. We will then measure levels of ETrA/B mRNA via RT-PCR and protein via Western analysis at 4, 24 and 48 hours post TBI to determine whether ETrA antagonists have any effect on ETrA/B message or protein levels.

Experiment 5.2: Determine the effect of BQ-788 or Bosentan on levels of ETrA/B mRNA and protein after TBI.

Here we will proceed as in **5.1**, however we will use the ETrB antagonist BQ-788 or the mixed antagonist Bosentan.

Experiment 5.3: Determine the effect of injection of BQ-123 followed by BQ-788 on CBF after TBI.

Here we will administer TBI. 30 minutes later we will inject BQ-123 as in experiment **3.1**. However to test whether there is a reciprocal effect of ETrA/B we will then administer BQ-788 at 12 hours post TBI. We will measure CBF at 4, 24 and 48 hours post TBI to determine whether blocking ETrB ameliorates the improved CBF seen with administration of ETrA antagonists only.

Experiment 5.4: Determine the effect of injection of BQ-788 followed by BQ-123 on CBF after TBI.

To further test reciprocal relationships between ETrA/B, we will proceed as in **5.3**, however we will administer the ETrB antagonist first followed by BQ-123.

Experiment 5.5: Determine the effect of injection of BQ-123 followed by BQ-788 on cognitive behavior after TBI.

To establish the functional outcome of experiment **5.3**, we will repeat this experiment, however test for behavioral outcome as before.

Experiment 5.6: Determine the effect of injection of BQ-788 followed by BQ-123 on cognitive behavior after TBI.

To establish the functional outcome of experiment **5.4**, we will repeat this experiment, however test for behavioral outcome as before.

Expected Results: Based on the provided preliminary data, we predict that while BQ-788 has no effect on either ETrA or B expression, BQ-123 causes an upregulation of ETrB. We also predict that as ETrB mediates vasodilation, by blocking this receptor after BQ-123, we would block the ability of BQ-123 to ameliorate hypoperfusion. Following the logic that sustained decreased blood flow exacerbates neuronal injury and causes cognitive deficits, we also predict, as our preliminary data suggests, that using BQ-788 after BQ-123 would prevent the improved cognition seen after TBI.

Possible pitfalls, alternative approaches: While interactions between ETrA/B have been only alluded to (Begnini et al., 2007) and mixed antagonists have been shown to have little clinical efficacy, to date few studies have been designed to tease out potential functional relationships between ETrA and B. Furthermore, no one has tested these interactions following TBI. While we acknowledge that this AIM is based almost entirely on our own observations, based on the strength of our preliminary data, we feel confident that the anticipated data not only will enhance our understanding of how ETrA/B interaction controls CBF but also how this interaction may be altered following brain trauma. Knowledge obtained from this set of experiments, in turn, can be used to more effectively design strategies utilizing endothelin antagonists in the clinical setting.

Experiment 5.7: *Determine the effect BQ-123 or Clazosentan on ET-1 mediated hypoperfusion in the absence of TBI on ETrA/B protein expression.*

*Here we will administer an ICV injection of ET-1 as in AIM 1 in order to achieve approximately a 35% decrease in CBF. 30 minutes after injection we will administer either BQ-123 or Clazosentan as in Experiment **5.1**. We will then measure levels of ETrA/B mRNA via RT-PCR and protein via Western analysis at 4, 24 and 48 hours post TBI to determine whether ETrA antagonists have any effect on ETrA/B message or protein levels after ET-1 induced hypoperfusion.*

Expected Results: *Based on the provided preliminary data, we predict that ETrA antagonists will not have the same effect on ETrA/B levels following ET-1 injection as that seen following TBI.*

Possible pitfalls, alternative approaches: *We can best interpret this finding as showing that hypoperfusion, alone, does not account for ETrA antagonist-mediated changes in ETrB levels. Therefore, there must be another mechanism other than simply hypoperfusion that accompanies TBI which leads to said changes. Therefore we acknowledge that this finding opens up a whole new set of questions as to how exactly TBI causes ETrA antagonists to change ETrB expression. We also acknowledge that to answer these questions goes far beyond the scope of this proposal. However, we also would like to point out that this finding increases enthusiasm for using*

ETrA antagonists in the clinical setting since the changes in ETrB observed after antagonist injection could account for the longer-lasting effect (i.e., one dose is sufficient to ameliorate changes in CBF).

NOTE: While we have evidence suggesting that the ET-1 response after TBI is limited to the brain (see preliminary results), we are well aware that injecting ETrA/B antagonists IV may have effects in the periphery. Since the overall goal is to determine a drug regime that most effectively treats some of the symptoms of TBI without having deleterious side-effects, we will measure mean arterial pressure (MAP) in ALL animals after injection to ensure that any potential positive results in the brain are not confounded by adverse effects in the periphery.

GENERAL METHODS

Closed Head Trauma Model:

Adult male Sprague-Dawley rats (300-375g) (Harlan Industries) will be anesthetized with 5% halothane in 2% oxygen prior to intubation, and then maintained on 1.5% halothane via a mask and spontaneous breathing. Halothane will be used as the anesthetic for all experiments. The use of halothane instead of the more recently introduced isofluorane is preferred because of recent evidence indicating the latter neuroprotective properties (published data [Zhao et al., 2007; Wei et al., 2007] and more recent data presented at the Brain '07, International Cerebral Blood Flow and Metabolism meeting held in Osaka, Japan). A midsagittal scalp incision will be performed and the underlying muscles retracted laterally. Cranioplastic cement will be used to attach a 10mm diameter X 3 mm thick, round metal helmet directly to the skill over the sagittal suture and between the coronal and lambdoidal sutures. The helmet is used to distribute the applied force over the surface of the parietal bones, thus preventing skull fractures with penetrating brain injury. After the cement is allowed to dry for three minutes, the animals will be placed prone on a platform as described in the Acceleration Impact Trauma Model of Marmarou (Marmarou et al., 1994). After 30-40 seconds of placement, 450g of weight contained in a hollow plastic cylinder will be dropped directly onto the helmet from a height of 2 meters. Following a brief convulsion and respiratory arrest, most animals restart breathing on their own. However, in some cases, the use of a rodent respirator or CPR is necessary prior to spontaneous breathing. Using these precautions, mortality has been reduced to less than 5%. In some animals after impact, the helmet will be removed and the skin sutured only if the skull shows no evidence of fractures. After suturing the skin, sensory cutaneous and evoked motor responses will be tested. Usually the intubation tube is removed at 10 minutes post trauma and only animals which are able to right themselves before 30 minutes after injury will be included in the study (Petrov et al., 2000; Petrov et al., 2002a). Brain and leg muscle temperatures will be taken routinely, in some instances up to 24 hrs post injury. We have determined that brain temperature fluctuated only 1.5°C, and muscle temperature 1.3°C, during this time period.

IV v ICV:

All receptor antagonist delivery will be performed by ICV and intravenous (IV) injection of the agent. This will be done so that we can compare local brain effects to those that include potential systemic effects so as to provide data which can be quickly incorporated into the clinical setting. Only in AIM 1 the ET-1 will be injected into the brain due to the known effects of ET-1 on the periphery. It is known that all other agents do not cross the blood-brain-barrier. We will use this to our advantage to rule out non-vascular ETrA/B, the function of which is not known.

CBF Measurements:

Prior to image acquisition, anesthesia will be induced by a steady application of 1% halothane using a specially designed apparatus compatible with the MRI to sedate the animals. The animal will be placed in a prone position on a cradle with a custom-built palate holder equipped with an adjustable nose cone and stereotaxic ear bars in order to minimize movement during MRI scans.

The rat head will be positioned at the isocenter of a magnet. MRI scans will be repeated at four time points. Baseline scans will be run before TBI is induced, and then at the 4th hour, 24th hour and 48th hour post-TBI. All MRI measurements will be performed on a 4.7-T horizontal-bore magnetic resonance spectrometer (Bruker AVANCE) with an 11.6-cm-bore actively shielded gradient coil set capable of producing a magnetic field gradient up to250 mT/m. A whole-body birdcage radiofrequency (RF) coil (inner diameter, 72 mm) will be used as the transmitter for homogeneous RF excitation, and a surface coil (30 mm diameter) will be used as the receiver, with active RF decoupling to avoid signal interference. Four sequences will be run in this set of experiments: T2-weighted imaging, T1-weighted imaging, and ASL for the measurement of flow and SWI to measure changes in oxygen saturation and flow, as well as for the determination of evidence of vascular damage and hemorrhage.

For all sequences, the field of view will be 40X40X24 mm^3; thus, the whole brain will be imaged. The remaining imaging parameters used are as follows:T2-weighted imaging Rapid Acquisition with Relaxation Enhancement (RARE): TR=4751 ms, TE=46 ms, matrix size NxXNy =256X256, number of slices (Ns)=24 (thickness, 1 mm),

Nacq=1; T1-weighted imaging (3D Fast Low Angle Shot [FLASH]): TR=22 ms, TE=7 ms, flip angle (FA)=58 and 208, matrix size NxXNyXNz =256X256X24, Nacq=1(FAs 58 and 208 will be used to calculate T1 maps); ASL: TR = 1550 ms, TE = 7.65 ms, matrix size NxXNy =128X70 (interpolated by zero filling in k-space to 256_256), slice=1 (thickness, 2 mm), Nacq=2, labeling slice=2 cm offset from isocenter, adiabatic fast passage with Magnetization transfer contrast (MTC) gradients=1.5 s, spin echo=3; SWI: TR=36 ms, TE=15 ms, FA=208, matrix size NxXNyXNz =512X512X24, Nacq=2. SWI is based on a fully flow-compensated, high-resolution, 3D gradient-echo method [20]. Flow compensation ensures that there is no flow-induced phase in SWI-filtered phase images.

Extraction of SM/EN from whole brain preparations:
In all cases, gene and protein levels will be measured in the areas specified in each AIM in tissue containing neurons, neuropil, vascularization, etc. and this will be compared with data taken from just the vascularization. In order to assess gene and protein levels in vascularization we will first remove brain and cut 2-mm thick slices containing the region of interest. Next we gently remove meninges, collect cortex and subcortex cut tissue into 1 mm segments and homogenize tissue in 3 ml ice-cold PBS using a Dounce homogenizer (30 strokes for loose grinder and 25 strokes for tight grinder). Then we spin for 3500 g for 10 min at 4 ^{0}C, then resuspend in 3 ml PBS. Next we filter the tissue suspension through a 41-□m nylon mesh (Spectrum), wash the mesh three times with 5 ml PBS. We usually use a small vacuum pressure to help the filtration). Microvessels are retained on the mesh, and so we wash them off with PBS, spin 3000 g for 10 min at 4 ^{0}C to pellet the microvessels and re-suspend the pellet in 15% dextran T-500, applying the suspension onto 20% dextran T-500. Finally, we spin at 25,000g for 10 min, collect the pellet as microvessels.

Real-time PCR:
Real-time PCR following a previously optimized protocol (Guo et al., 2007) will be used to determine gene expression. Briefly, total RNA is isolated by using STAT-60 Reagent (Tel-Test Co., TX, USA) according to the manufacturer's protocol. Next, the samples are purified using the RNeasy kit (RNeasy Mini Kit, Qiagen, MD, USA) and DNase Treatment & Removal Kit (Ambion, CA, USA). Random primers are used to create first-strand DNA synthesis using iScript cDNA synthesis kit (Bio-Rad). The cDNA is then amplified using an ABI Prism 7900HT sequencing detection system for real-time PCR with SYBR Green PCR Master Mix (Applied Biosystems, CA, USA). To determine the relative quantification of target gene expression, we used the comparative CT (threshold cycle) method with arithmetic formulae (Heid et al., 1996, Gibson et al., 1996, Hiratsuka et al., 1999; Ding et al., 2003).

Western Analysis:
Whole brains will be harvested (n=4 animals per group), placed in cold methylbutane on dry ice and partially frozen. Brains will then be dissected to isolate smCx and hipp. Isolated smCx and hipp will be homogenized in Lamelli's solution and subjected to SDS-PAGE. Protein concentrations will be standardized for all samples. Electrophoresis will run at 40mA for the first 40 min and then 20mA for 3 hrs. Gels are transferred to nitrocellulose paper, and blocked using 1% nonfat dried milk in TTBS at room temperature for one hour. Transferred samples will then be incubated in primary antibody (individual antibodies listed as needed per experiment) in TTBS at 4^{0}C overnight and then incubated in secondary antibody and donkey serum in TTBS at room temperature for 30 min. Nitrocellulose will be rinsed in Lumiglow solution for 90 seconds, exposed to X ray film and developed. Band intensity of immunoblots will be quantified using optical densitometric (OD) analysis.

ICC and Double Immunofluorescence:
Multiple fluorescence labeling has been used effectively by this laboratory in the past 20 years to reveal alterations in CNS after injury (Petrov et al., 1991; 1992; 2000) and therefore we do not anticipate any caveats (also see Preliminary Data). Multiple series of coronal sections (40 µm thick) through the hippocampus and striatum will be cut on a cryostat and collected in 1% PBS as free-floating sections. The series consist of tissue from each group of animal and will be used for detecting the following: EN+ETrA or B, SMA+ETrA or B, GFAP+ETrA or B, NeuN+ETrA or B. Tissue will first be permeabilized with 0.6% Triton X. The monoclonal antibody generated against BrdU is conjugated to FITC (1:150; Sigma, St. Louis, MO). The lectin (Banderiaes simplicifolia, 1:100; Sigma) is conjugated with FITC and was previously used by us to visualize En membranes. Anti-SMA (α-smooth muscle actin) is also FITC-conjugated. GFAP is used to detect astroglia and is conjugated to AMCA; NeuN is used to detect neurons and is also AMCA-conjugated. ETrA or rB will be detected using monoclonals raised in mouse (1:100; Sigma). For visualization, antibodies are biotinylated using a Vectastain kit (1:100; Vector Laboratories, Burlingame, CA, USA) followed by Texas Red (TR) conjugated to streptavidin (1:100; Jackson Immuno Research Laboratories, Westgrove, PA, USA). Control sections omitting primary antibodies were used to assess non-specific binding.

Assessment of motor deficit:

Since neurological deficits, while rarely seen using this model of TBI, would greatly hinder the ability of a rat to perform on the radial arm maze or in a Morris Water Maze, all animals will be screened following TBI for neurological outcome. In order to screen animals for motor deficit, all TBI animals will be tested using standard neurological function tests, including rotor rod performance, balance beam, and ladder climbing. Based on preliminary screens, rats either performed well or, on the contrary, showed deficit on all tests and, therefore, animals performing at sub-control levels on any test will be grounds for removal from the study.

Behavioral Testing and Radial Arm Maze Setup:
The rats will be allowed to acclimate to their new environment (in DLAR facility) after their arrival. Then from day 1 to day 3 of the behavioral study the rats will be handled by the researcher for 10 to 15 minutes each. Acclimation to the maze environment also will be initiated during which the rats will be placed on the central platform of the radial arm maze and allowed to roam freely.

A custom designed radial arm maze will be built using black acrylic sheet (0.6 cm thick). Eight identical radial arms are fixed to an octagonal base platform that stands 63 cm above the floor. Each radial arm is 60 cm in length and 10 cm in width with 10 cm – high sidewalls along each arm. At the end of each arm a 5-cm end piece is placed. A hole measuring 2.5 cm in diameter is also cut 5 cm from the end of each radial arm to place a plastic food cup (1 oz).

During behavioral testing, the maze is enclosed within four black linen walls. A white paper triangle (15-cm sides) is placed on one linen wall 10 cm above the base of radial arm #3. An 8" x 11" white paper square with bisecting black lines is placed on the same linen wall 10 cm above the base of radial arm #5. A researcher holding a green notebook and always wearing a bright yellow surgical gown with latex gloves will be positioned in front of radial arm #8 during the study. These three visual cues are aimed to provide spatial guidance as to the location of the baited arm (i.e. containing the food).

Radial arm maze trials will commence from the 3rd day of the behavioral study. The rats will be tested for the time taken to find the bait (half of a Fruit Loop cereal®) placed in a plastic cup of four different radial arms. Each rat will be tested daily for three consecutive time trials. The maximum time a rat will be allowed to spend in the maze is ten minutes by the end of which determined to be conclusion of a trial. Averages of these trials will be calculated and recorded.

Electron Microscopy (EM):
Vasoreactivity will be determined using EM and measurement of luminal area as previously described (Rafols et al., 2007). Briefly sham-operated controls and experimental (post ET-1 injection at various time intervals) animal groups (n=4 animals/group) will be anesthetized with 2.5% halothane. Animals will be perfused through the ascending aorta with isotonic saline (50 cc) followed by a 200 cc solution containing 4% paraformaldehyde and 0.1% gluteraldehyde in 0.1 M phosphate-buffered saline (PBS). Following perfusion, each brain is carefully resected from the skull and stored in the same perfusion fluid at 4^{0}C overnight. Each brain is then sectioned coronally at 300 µm with a vibratome. Sections containing the sensorimotor cortex (-1.5 to -3.5 mm from bregma, Paxinos and Watson,1998) are further trimmed by two consecutive vertical cuts from the pial surface to the subcortical white matter, and a longitudinal cut immediately below the cortical gray matter. Isolated cortical blocks are then postfixed in 1% osmium tetroxide, dehydrated and plastic embedded for ultrathin and semithin (1.0µm) sectioning. Ultrathin sections are stained with uranyl acetate and lead citrate and examined with a JEOL JEM-1010 electron microscope.

One µm thick, plastic embedded sections
1.0 µm thick, toluidine blue-stained plastic sections are obtained from the plastic embedded blocks prior to the ultrathin sections for purposes of block orientation and identification of cortical layers. For quantification of luminal area, twenty five cross-sectioned, EM profiles of microvessels, including capillaries and small arterioles, at a total magnification of X25,000 will be selected from each animal (n=4 animals /group, as above). Profiles of venules are distinguished from those of capillaries on the basis of luminal size, although we acknowledge that it is difficult to identify the transition from a capillary to a vein. Care is taken that the selected profiles are sectioned at nearly perpendicular or at right angles to the long axis of the vessel (i.e., endothelial membrane sectioned mostly perpendicularly to the plane of section). The luminal area (µm^2) is then measured by using the Bioquant IV morphometric system integrated to an IBM-XT computer (R & M Biometrics). Average luminal area/animal group is then calculated by pooling the data from all the microvessels within a group. After data gathering, values between groups are compared using a one-way analysis of variance (ANOVA) test. Where the test revealed significant differences, the least significant difference post-hoc test is used to determine which groups are different. Significance is established at $P<0.05$.

Stereological quantitative analysis of luminal area:

The dissector, an unbiased stereological method (Gundersen, 1988), will be used to determine total luminal area. The dissector consists of two parallel EM sections of know thickness. One of the sections is referred to as the look-up section, whereas the other section is referred to as the reference section. Counting frames are placed over both sections. Any lumen that appears on the reference section, but not the look-up section is counted. The number of counted lumen, Q-, is an unbiased estimate of the number of lumen in the dissector. To determine area fraction (Aa), the counting frame with 121 evenly spaced points (Psect) is placed over both the look-up section and the reference sections and the points that hit the structure (Pstruc) are counted. Aa is derived from the following equation: Aa= Pstruc/Psect = Pstruc/121 and total area = Aa/Q-.

Statistical Analysis

CBF measurements. All data pertaining to CBF are expressed as the average of scans taken independently. CBF is expressed as mL/100g/min. Between group analyses are accomplished using one-way analysis of variance (ANOVA) with least significant difference (LSD) post-hoc testing. Data are reported as mean ± SE. Significance is set at p-value < 0.05. As previously reported (Shen et al., 2007), we were able to detect significant changes in CBF between groups using 6 animals per group with 95% power at $\alpha = 0.05$.

Western Analysis. All data pertaining to Western analysis of protein expression are expressed as the average of samples blotted independently. Between group analyses are accomplished using one-way analysis of variance (ANOVA) with least significant difference (LSD) post-hoc testing. Data are reported as mean ± SE. Significance is set at p-value < 0.05. As previously reported (abstract in 2006 Society for Neuroscience Annual Meeting, Atlanta, GA; Kreipke 2007c), we were able to detect significant changes in protein expression between groups using 4 animals per group with 95% power at $\alpha = 0.05$.

Immunofluorescence and immunocytochemistry. All histological and immunocytochemical analyses are conducted using 4 to 6 sections per animal with 3 to 6 areas of analysis per section (see methodology). Data is expressed as an average of each area of analysis. In between group analysis is accomplished using one-way analysis of variance (ANOVA), with least significant difference post-hoc testing. Data are reported as mean ± SE. Significance is set at p-value < 0.05. Based on variability in these data from our previous studies (Kreipke et al., 2006, 2007,a,b) we can distinguish a difference in individual proteins and in capillary density with 95% power at an α level of 0.05 with 4-6 rats per group.

Behavioral Assessments. All behavioral data are expressed as the average latency of completing the task over three trials. Between group analyses are accomplished using one-way analysis of variance (ANOVA) with least significant difference (LSD) post-hoc testing. Data are reported as mean ± SE. Significance is set at p-value < 0.05. Due to the variability in behavior amongst individual animals, we have previously determined (Kreipke 2004, 2007) that we can distinguish significant differences between TBI and control animals using 12 animals per group. Antagonist and agonist studies will require 12-15 animals per group to show an improved performance with power of 90%. This allows us to distinguish a difference in latency of 2 min with 90% power at $\alpha = 0.05$. Additional rats may be required due to failure to exercise, death, or motoric disability following injury.

Project Tentative Schedule:

YEAR	**1**	**2**	**3**	**4**	**5**
TASKS	AIM 1: test effects of ET-1 on vascular tone, CBF, ETrA/B expression, and behavioral outcome	AIM 1 and 2: test effects of ETrA/B antagonists on ET-1 effect on vascular tone, CBF, ETrA/B expression, and behavioral outcome; test effects of anti-Cp on cell injury and behavior	AIM 3: dose response and time course of BQ-123/ Clazosentan; effect on CBF, receptors and behavior	AIM 4: dose response and time course of BQ-788/ Bosentan; effect on CBF, receptors, and behavior	AIM 5: Effect of antagonists on receptor shifts; begin preliminary data for competing renewal (possible clinical trial)

Future Directions: Clinical trial; As stated in the background and significance section, Benigni and Remuzzi (1999) published one of the first works suggesting that specific ETrA antagonists may provide hope in the clinical setting in ameliorating vascular dysfunction in multiple pathological states. **We have already opened dialogue with Dr. Benigni to join her team in Bergamo, Italy in developing a clinical trial testing the effectiveness of using ETrA antagonists such as clazosentan in the treatment of vascular dysfunction following TBI.** If successfully funded, this work will provide the pre-clinical data needed to initiate this collaboration, which will quickly translate this work into the clinical realm.

Manuscripts published or in press under the funding period of the current grant

1. Rafols D, Steiner J, Rafols JA, Petrov T. 2004. Intracellular coexpression of endothelin-1 and inducible nitric oxide synthase underlies hypoperfusion after traumatic brain injury in the rat. Neurosci Lett 362:154-157.
2. Steiner J, Rafols D, Park HK, Katar MS, Rafols JA, Petrov T. 2004. Attenuation of iNOS mRNA exacerbates hypoperfusion and upregulates endothelin-1 expression in hippocampus and cortex after brain trauma. Nitric Oxide 10:162-169.
3. Kreipke CW, Morgan N, Petrov T, Rafols J. 2006. Calponin and caldesmon cellular domains in reacting microvessels following traumatic brain injury. Microvascular Research 71:197-204.
4. Shen Y, Kou Z, Kreipke CW, Petrov T, Hu J, Haacke EM. 2007. In vivo measurement of tissue damage, oxygen saturation changes and blood flow changes after experimental traumatic brain injury in rats using susceptibility-weighted imaging. Magn Reson Imaging 25:219-227.
5. Kreipke CW, Morgan R, Petrov T, Rafols JA. 2007. Subcellular Redistribution of Calponin Underlies Sustained Vascular Contractility Following Traumatic Brain Injury. Neurological Research 29:604-609.
6. Rafols JA. Microvascular and neuronal responses in a model of diffuse brain injury: therapeutic implications. Neurological Research 29:337-339.
7. Rafols J., Kreipke CW, Petrov T. 2007. Alterations in Cerebral Cortex Microvessels and the Microcirculation in a Rat Model of Traumatic Brain Injury: a Correlative EM and Laser Doppler Flowmetry Study. Neurological Research 29:339-347.
8. Rafols J, Morgan R, Kallikuri S, Kreipke CW. 2007. Extent of nerve cell injury Marmarou's model compared to other brain trauma models. Neurological Research 29:348-355.
9. Degracia D, Kayali F, Kreipke CW, Rafols JA. 2007. Brain endothelial HSP-70 stress response coincides with endothelial and pericyte death after brain trauma. Neurological Research 29:356-361.
10. Kallukuri S, Kreipke CW, Rossi N., Rafols JA, Petrov T. 2007. Spatial alterations in endothelin receptor expression are temporally associated with the altered microcirculation after brain trauma. Neurological Research 29:362-368.
11. Kreipke CW, Morgan R, Roberts G, Bagchi M, Rafols JA. 2007. Calponin phosphorylation in cerebral cortex microvessels mediates sustained vasoconstriction after brain trauma. Neurological Research 29:369-374.
12. Kreipke CW, Morgan R, Kallikuri S, Rafols J. 2007. Behavioral preconditioning enhances angiogenesis and cognitive outcome following traumatic brain injury. Neurological Research 29:388-394.
13. Huttemann M, Lee I, Kreipke CW, Petrov T. 2007. Suppression of iNOS prior to traumatic brain injury improves cytochrome oxidase activity and normalizes cellular energy levels. Neuroscience Sept (Epub ahead of print).
14. Kreipke CW, Petrov T, Rafols JA. (in press). Endothelin A receptor antagonism blocks calponin phosphorylation following brain trauma. J Cereb Blood Flow and Metab.
15. Rafols JA, Kreipke CW, Schafer PC, Michael D (in press). Endothelin receptors A and B are expressed in distinct cellular compartments of rat hippocampus following global ischemia: an immunocytochemical study. Canadian Journal of Physiology and Pharmacology.

Related Abstracts

1. Kreipke C, Petrov T., Rafols J. (2005) Upregulation and migration of calponin following traumatic brain injury. 35th Society for Neuroscience Abstracts 30 (Washington DC, USA).
2. Kreipke C, Petrov T. (2005) Interactions between endothelin (ET-1) and its receptors in the control of the brain microcirculation following traumatic brain injury (TBI). Salt Lake City, Utah. Endothelin 2005.
3. Kreipke C, Rafols J, Petrov T. (2005) Transcriptional and translational mechanisms for the reciprocal control of INOS and endothelin 1 expression in brain microvessels after traumatic brain injury. Amsterdam, Holland. 4.Brain05.000711.
4. Haacke M, Kreipke C, Morgan N, Shen Y, Hu J, Petrov T. (2006) Non-Invasive In Vivo Measurement of Cerebral Blood Flow and Oxygen Saturation Changes in Rat Brain after Trauma. International Neurotrauma Meeting (Rotterdam. Holland).

5. Kreipke CW, Morgan R, Petrov T, Rafols JA. (2006) Phosphorylation of calponin underlies sustained contractility following traumatic brain injury. 36th Annual Society for Neuroscience Meeting (Atlanta, GA, USA)
6. Morgan R, Kreipke CW, Petrov T, Rafols JA. (2006) Behavioral conditioning induces angiogenesis and improves cognitive and neurological outcome. 36th Annual Society for Neuroscience Meeting (Atlanta, GA, USA)
7. Shen Y, Kou Z, Hu J, Kreipke CW, Petrov T, Haacke EM. (2006) Non-invasive in vivo measurement of cerebral blood flow and oxygen saturation changes in rat brain after trauma. J Neurotrauma 23:789-789.
8. Morgan N, Kreipke C, Rafols J, Petrov T. (2006) Alterations in Caldesmon Expression Following Traumatic Brain Injury. National Conference on Undergraduate Research (NCUR) (Asheville, NC, USA).
9. Kreipke CW, Morgan R, Rafols JA. (2007) Ultrastructural and subcellular changes in vascular wall of cerebral microvessels following traumatic brain injury. 1st Annual International Brain Conference (Orlando, FL, USA)
10. Kreipke CW, Morgan R, Rafols JA. (2007) Endothelin receptor antagonism blocks calponin phosphorylation following traumatic brain injury. To be presented at the International Brain/Brain Pet Meeting '07 (Osaka, Japan)
11. Kreipke CW, Schafer PC, Bedford C, Petrov T, Rafols JA. (2007) Endothelin receptor A antagonism ameliorates hypoperfusion and leads to improved performance on a radial arm maze following traumatic brain injury (TBI). ET-10 panel discussion, ET-10, Bergamo, Italy.
12. Kallakuri S, Kreipke CW, Petrov T, Rafols JA. (2007) Endothelin receptors are localized in different brain cell types after traumatic brain injury. 37th Society for Neuroscience Meeting (San Diego, CA, USA).
13. Kreipke CW, Kallakuri S, Rafols JA, Petrov T. (2007) The role of endothelin receptors in the altered microcirculation after traumatic brain injury (TBI): an in situ hybridization study. 37th Society for Neuroscience Meeting (San Diego, CA, USA).

V. VERTEBRATE ANIMALS

The choice to use male Sprague-Dawley rats is based on previous work both in our lab and in the labs cited in the research design. No more than 293 male rats will be used in total (see below). Further, rats will be used because of their low cost and because of the large body of information that is now known about their basic neuroanatomy, physiology, and behavior. Rats have an extremely high resistance to infection and are small in size which precludes using large amounts of expensive agents. In addition, the Sprague-Dawley strain has been shown to display pathological changes comparable to those encountered in clinical conditions. Adherence to IACUC guidelines will be maintained in the experimental treatment and housing of the animals. Housing is provided in an IACUC approved facility in the same buildings as the laboratories (Dr. Kreipke's laboratory and the Department of Animal Laboratory Research Testing Facility). Training in proper care and handling of animals, as provided by the Wayne State University Department of Laboratory Animal Resources, has been successfully completed by the applicant.

Brief Summary of Procedures: After brain injury, some animals may experience persisting respiratory difficulties, and will be ventilated as necessary. If this ailment lasts longer than 60 min, such animals will be euthanized with sodium pentobarbital (120 mg/kg, IP injection) consistent with our previous work and with the Panel on Euthanasia of the American Veterinary Medical Association. It is possible that some degree of pain and distress will be present as a consequence of impact on the skull. However, animals are typically awake, but quiet and relatively inactive after trauma. By 1 hour they are usually active and are capable of eating and drinking on their own, although a drop of approximately 7% in body weight is expected. Analgesics will not be used immediately after injury because they (1) interfere with measurements of cerebrovascular function, (2) have neuroprotective effects and (3) in our experience with humans, there is very little or no need for analgesics right after a severe head injury. The effects of analgesics would compromise the results from the proposed experiments. Upon termination of a given testing period, rats will be euthanized with a lethal dose of sodium pentobarbital (120 mg/kg IP as above) and death will be assured by bilateral pneumothorax and severing the aorta.

Number of Animals: The number of rats required is based on power studies for the protocols (see Statistics) and on anticipated loss/exclusion of animal subjects due to technical issues such as death upon impact (~10%), motor deficit following injury (~10%), malposition or clogging of cannulae (~5%), or failure to thrive (~2%). Thus, approximately 25% more rats will need to be entered into the studies for adequate power to be achieved. Note that every attempt has been made to minimize control animals by comparing data across experiments (see experimental design for more detail).

Specific Aim 1
Experiment 1-1a: 4 groups (3 doses, 1 veh) X 6 = 24
Experiment 1-1b: 2 groups (BQ-123 v. veh) X 6 = 12
Experiment 1-1c: 2 groups (BQ-788 v. veh) X 6 = 12
Experiment 1-2a: 2 groups (ET-1 v. veh) X 3 = 6
Experiment 1-2b: 2 groups (BQ-123 v. veh) X 6 = 12
Experiment 1-2c: 2 groups (BQ-788 v. veh) X 6 = 12
Experiment 1-3: 2 groups (ET-1 v. veh) X 4 = 8
Experiment 1-4a: 2 groups (ET-1 v. veh) X 12 = 24
Experiment 1-4b: 2 groups (BQ-123 v. veh) X 15 = 30
Experiment 1-4c: 2 groups (BQ-788 v. veh) X 15 = 30

Specific Aim 2
Experiment 2-1: 7 groups (TBI only v. veh v. various doses v. sham-operated) X 8 = 56
Experiment 2-2: 3 time points X 8 = 24

Experiment 2-3: 2 groups (BQ-123 v. veh) X 12 = 24

Specific Aim 3
Experiment 3-1: 7 groups (TBI only v. veh v. various doses v. sham-operated) X 8 = 56
Experiment 3-2: 3 time points X 8 = 24
Experiment 3-3: 2 groups (BQ-788 v. veh) X 12 = 24

Specific Aim 4
Experiment 4-1: 6 groups (5 doses, veh) X 4 = 24
Experiment 4-2: 6 groups (5 doses, veh) X 6 = 36
Experiment 4-3: 3 time points X 6 = 18
Experiment 4-4: 2 groups (Clazosentan v. veh) X 15 = 30

The total number of rats for all protocols is 486. However we will be prepared to include up to 122 more to account for the 25% loss/exclusion rate for a total of 608 animals.

References Cited

Arai H, Hori S, Aramori I, Ohkubo H, Nakanishi S. (1990) Cloning and expression of a cDNA encoding an endothelin receptor. Nature. 348:730-732.

Baldys-Waligórska A, Szybiński Z. (1992) Plasma endothelin 1/2 levels in healthy blood donors as measured by RIA--a clinical application. Endokrynol Pol. 43(1):7-12.

Baldys-Waligórska A, Szybiński Z. (1993) Plasma endothelin 1/2 levels in healthy blood donors and in hypertensive patients: clinical application. Endocr Regul. 27(2):83-7.

Bárány K, Polyák E, Bárány M. (1992) Involvement of calponin and caldesmon in sustained contraction of arterial smooth muscle. Biochem Biophys Res Commun. 187(2):847-52.

Barone FC, Ohlstein EH, Hunter AJ, Campbell CA, Hadingham SH, Parsons AA, Yang Y, Shohami E. (2000) Selective antagonism of endothelin-A-receptors imporves outcome in both head trauma and focal stroke in rat. J Cardiovasc Pharmacol. 36 (Suppl 1):S357-S361.

Bell KM, Chaplin D, Poole BA, Prise VE, Tozer GM. (1999) Modifications of blood flow in the HSN tumor and normal tissues of the rat by the endothelin ET (B) receptor agonist IRL 1620. Int J Cancer. 80:259-302.

Benigni A, Remuzzi G. (1999) Endothelin antagonists. Lancet. 353(9147):133-8.

Bousso-Mittler D, Bdolah A, Sokolovsky M. (1989) Three apparent receptor subtypes for the endothelin/sarafotoxin family. FEBS Lett. 253:199-202.

Cazaubon S, Chaverot N, Romero IA, Girault JA, Adamson P, Strosberg AD, Couraud PO. (1997) Growth factor activity of endothelin-1 in primary astrocytes mediated by adhesion-dependent and –independent pathways. J Neurosci. 17:6203-6212.

Cazabon SM, Couraud PO. (1998) Nitric oxide and endothelin at the blood brain barrier. In: Introduction to the blood brain barrier: Methodology, Biology, Pathology (Pertridge WM, ed.), 338-344. New York: Cambridge University Press.

Clozel M, Gray GA, Breu V, Loffler BM, Osterwalder R. (1992) The endothelin ETB receptor mediates both vasodilation and vasoconstriction in vivo. Biochem Biophys Res Commun. 86:867-873.

Clozel M, Watanabe H. (1993) BQ-123, a peptidic endothelin ETA receptor antagonist, prevents the early cerebral vasospasm following subarachnoid hemorrhage after intracisternal but not intravenous injection. Life Sci. 52:825-834.

Dawson DA, Sugano H, McCarron RM, Hallenbeck JM, Spatz M. (1999) Endothelin receptor antagonist preserves microvascular perfusion and reduces ischemic brain damage following permanent focal ischemia. Neurochem Res. 24(12):1499-505.

Degracia D, Kayali F, Kreipke CW, Rafols JA. 2007. Brain endothelial HSP-70 stress response coincides with endothelial and pericyte death after brain trauma. Neurological Research 29:356-361.

Ding Y, Young CN, Li J, Luan X, McAllister JP 2nd, Clark JD, Diaz FG. (2003) Reduced inflammatory mediator expression by pre-reperfusion infusion into ischemic territory in rats: a real-time polymerase chain reaction analysis. Neurosci Lett. 353(3):173-6.

D'Orleans-Juste P, Lidbury PS, Warner TD, Vane JR. (1990) Intravascular big endothelin increases circulating levels of endothelin-1 and prostaglandins in the rabbit. Biochem Pharmacol. 39:r21-22.

Epstein M. (1994) Hepatorenal syndrome: emerging perspectives of pathophysiology and therapy. J Am Soc Nephrol. 4(10):1735-53.

Fukuroda T, Fujikawa T, Ozaki S, Ishikawa K, Yano M, Nishikibe M. (1994) Clearance of circulating endothelin-1 by ETB receptors in rats. Biochem Biophys Res Commun. 199:1461-1465

Gibson UE, Heid CA, Williams PM. (1996) A novel method for real time quantitative RT-PCR. Genome Res. 6(10):995-1001.

Gorlach C, Hortobagyi T, Hortobagyi S, Benyo Z, Wahl, M. (2001) Inhibition of endothelin-1 by the competitive ETA receptor antagonist Ro 61-1790 reduces lesion volume after cold injury in the rat. Pflugers Arch. 441:844-849

Granstam SO, Granstam E, Fellstrom B, Lind L. (1998) Effects of endothelin receptor type A ET-RA and ET-RB antagonism and nitric oxide synthase inhibition on cerebral blood flow in hypertensive rats. Acta Physiol Scand. 164:213-218.

Gundersen HJ. (1988) The nucleator. J Microsc. 151(Pt 1):3-21.

Guo F, Chiang MY, Wang Y, Zhang YZ. (2007) An in vitro recombination method to convert restriction- and ligation-independent expression vectors. Biotechnol J. E-Pub.

Hama H, Kasuya Y, Sakurai T, Yamada G, Suzuki N, Masaki T, Goto K. (1997) Role of endothelin-1 in astrocytes responses after acute brain damage. J Neurosci Res. 47:590-602.

Han H, Neubauer S, Braeker B, Ertl G. (1995) Endothelin-1 contributes to ischemia/reperfusion injury in isolated rat heart-attenuation of ischemic injury by the endothelin-1 antagonists BQ123 and BQ610. J Mol Cell Cardiol. 27:761-766.

Harrison VJ, Randriantoa A, Schoeffter P. (1992) Heteroge Pharm Neity of endothelin-sarafotoxin receptors mediating contraction of pig coronary artery. Br J Acol. 105:511-513.

Hasunuma K, Rodman DM, O'Brien RF, McMurtry IF. (1990) Endothelin 1 causes pulmonary vasodilation in rats. Am J Physiol. 259(1 Pt 2):H48-54.

Heid CA, Stevens J, Livak KJ, Williams PM. (1996) Real time quantitative PCR.Genome Res. 6(10):986-94.

Hirata Y, Yoshimi H, Takata S, Watanabe TX, Kumagai S, Nakajima K, Sakakibara S. (1988) Cellular mechanism of action by a novel vasoconstrictor endothelin in cultured rat vascular smooth muscle cells. Biochem Biophys Res Commun. 154:868-875.

Hiratsuka M, Agatsuma Y, Mizugaki M. (1999) Rapid detection of CYP2C9*3 alleles by real-time fluorescence PCR based on SYBR Green. Mol Genet Metab. 68(3):357-62.

Hirose H, Ide K, Sasaki T, Takahashi R, Kobayashi M, Ikemoto F, Yano M, Nishikibe M. (1995) The role of endothelin and nitric oxide in modulation of normal and spastic cerebral vascular tone in the dog. Eur J Pharmacol. 277:77-87.

Ho MC, Lo AC, Kurihara H, Yu AC, Chung SS, Chung SK. (2001) Endothelin-1 protects astrocytes from hypoxic/ischemic injury. FASEB J. 15:618-626.

Hori S, Komatsu Y, Shigemoto R, Mizuno N, Nakanishi S. (1992) Distinct tissue distribution and cellular localization of two messenger ribonucleic acids encoding different subtypes of rat endothelin receptors. Endocrinology. 130:1885-1895.

Ihara M, Noguchi K, Saeki T, Fukuroda T, Tsuchida S, Kimura S, Fukami T, Ishikawa K, Nishikibe M, Yano M. (1992) Biological profiles of highly potent novel endothelin antagonists selective for the ETA receptor. Life Sci. 50:247-255.

Inoue A, Yanagisawa M, Kimura S, Kasuya Y, Miyauchi T, Goto K, Masaki T. (1989) The human endothelin family: three structurally and pharmacologically distinct isopeptides predicted by three separate genes. Proc Natl Acad Sci USA. 86:2863-2867.

Ishikawa K, Fukami T, Nagase T, Fujita K, Hayama T, Niiyama K, Mase T, Ihara M, Yano M. (1992) Cyclic pentapeptide endothelin antagonists with high ETA selectivity. Potency- and solubility – enhancing modifications. J Med Chem. 35:2139-2142.

Ishikawa K, Ihara M, Noguchi K, Mase T, Mino N, Saeki T, Fukuroda T, Fukami, Ozaki S, Nagase T, et al. (1994) Biochemical and pharmacological profile of a potent and selective endothelin B-receptor antagonist, BQ788. Proc Natl Acad Sci USA. 91:4892-4896.

Itoh S, Sasaki T, Ide K, Ishikawa K, Nishikibe M, Yano M. (1993) A novel endothelin ETA receptor antagonist, BQ-485, and its preventive effect on esperimental cerebral vasospasm in dogs. Biochem Biophys Res Commun. 195-969-975.

Ivy DD, Kinsella JP, Abman SH. (1994) Physiologic characterization of endothelin A and B receptor activity in the ovine fetal pulmonary circulation. J Clin Invest. 93(5):2141-8.

Josko J, Hendryk S, Jedrzejowska-szypulka H, Slowinski J, Gwozdz B, Lange D, Snietura M, Zwirska-Korczala K, Jochem J. (2001) Cerebral angiogenesis after subarachnoid hemorrhage (SAH) and endothelin receptor blockage with BQ-123 antagonist in rats. J Physiol Pharmacol. 52:237-248.

Kallukuri S, Kreipke CW, Rossi N., Rafols JA, Petrov T. (2007a) Spatial alterations in endothelin receptor expression are temporally associated with the altered microcirculation after brain trauma. Neurological Research 29:362-368.

Kallakuri S, Kreipke CW, Petrov T, Rafols JA. (2007b) Endothelin receptors are localized in different brain cell types after traumatic brain injury. 37th Society for Neuroscience Meeting (San Diego, CA, USA).

Kloog Y, Sokolovsky M. (1989) Similarities in mode and sites of action of sarafotoxins and endothelins. Trends Pharmacol Sci. 10:212-214.

Kreipke CW, Morgan N, Petrov T, Rafols J. (2006) Calponin and caldesmon cellular domains in reacting microvessels following traumatic brain injury. Microvascular Research 71:197-204.

Kreipke CW, Morgan R, Petrov T, Rafols JA. (2007c) Subcellular Redistribution of Calponin Underlies Sustained Vascular Contractility Following Traumatic Brain Injury. Neurological Research 29:604-609.

Kreipke CW, Morgan R, Roberts G, Bagchi M, Rafols JA. (2007a) Calponin phosphorylation in cerebral cortex microvessels mediates sustained vasoconstriction after brain trauma. Neurological Research 29:369-374.

Kreipke CW, Morgan R, Kallikuri S, Rafols J. (2007b) Behavioral preconditioning enhances angiogenesis and cognitive outcome following traumatic brain injury. Neurological Research 29:388-394.

Kreipke CW, Petrov T, Rafols JA. (in press, d) Endothelin A receptor antagonism blocks calponin phosphorylation following brain trauma. J Cereb Blood Flow and Metab.

Kreipke CW, Schafer PC, Bedford C, Petrov T, Rafols JA. (2007) Endothelin receptor A antagonism ameliorates hypoperfusion and leads to improved performance on a radial arm maze following traumatic brain injury (TBI). ET-10 panel discussion, ET-10, Bergamo, Italy.

Kreipke CW, Morgan R, Rafols JA. (2007) Endothelin receptor antagonism blocks calponin phosphorylation following traumatic brain injury. To be presented at the International Brain/Brain Pet Meeting '07 (Osaka, Japan)

Kreipke C, Petrov T. (2005) Interactions between endothelin (ET-1) and its receptors in the control of the brain microcirculation following traumatic brain injury (TBI). Salt Lake City, Utah. Endothelin 2005.

Kreipke CW, Walker PD. (2004) NMDA receptor blockade attenuates locomotion elicited by intrastriatal dopamine D1-receptor stimulation. Synapse 53:25-32.

Krep H, Brinker G, Schwindt W, Hossmann KA. (2000) Endothelin type A-antagonist improves long-term neurological recovery after cardiac arrest in rats. Crit Care Med. 28:2873-2880.

Krum H, Liew D. (2003) Current status of endothelin blockade for the treatment of cardiovascular and pulmonary vascular disease. Curr Opin Investig Drugs. 4(3):298-302.

Koyama Y, Baba A. (1996) Endothelin-induced cytoskeletal actin re-organization in cultured astrocytes: inhibition by C3 ADP-ribosyltransferase. Glia. 16(4):342-50.

Kurokawa K, Yamada H, Ochi J. (1997) Topographical distribution of neurons containing endothelin type A receptor in the rat brain. J Comp Neurol. 389:348-360.

Lazarini F, Strosberg AD, Couraud PO, Cazaubon SM. (1996) Coupling of ETB endothelin receptor to mitogen-actived protein kinase stimulation and DNA synthesis in primary cultures of rat astrocytes. J Neurochem. 66:459-465.

Lüscher TF, Wenzel RR. (1995) Mechanisms of acute coronary syndrome. Schweiz Rundsch Med Prax. 84(6):155-64.

MacCumber MW, Ross CA, Snyder SH. (1990) Endothilin in brain: receptors, mitogenesis, and biosynthesis in glial cells. Proc Natl Acad Sci USA. 87:2359-2363.

Marmarou A, Foda MA, Van Den Brink W, Campbell J, Kita H, Demetriadou K. (1994) A new model of diffuse axonal injury in rats. Part I: Pathophysiology and biomechanics. J. Neurosurg. 80:291-300.

Marsault R, Feolde E, Frelin C. (1993) Receptor externalization determines sustained contractile responses to endothelin-1 in rat aorta. Am J Physiol. 264:C687-693.

Marsault R, Vigne P, Breittmayer JP, Frelin C. (1990) Astrocytes are target cells for endothelins and sarafotoxin. J Neurochem. 54:2142-2144.

Masaki T. (1993) Endothelins: homeostatic and compensatory actions in the circulatory and endocrine systems. Endocr. Rev. 14:256-268.

McMahon EG, Palomo MA, Moore WM, McDonald JF, Stern MK. (1991) Phosphoramidon blocks the pressor activity of porcine big endothelin-1 in vivo and conversion of big endothelin-1 to endothelin-1 in vitro. Proc. Natl. Acad. Sci USA. 88:703-707.

Mino N, Kobayashi M, Nakajima A, Amano H, Shimamoto K, Ishikawa K, Watanabe K, Nishikibe M, Yano M, Ikemoto F. (1992) Protective effect of a selective endothelin receptor antagonist, BQ123, in ischemic acute renal failure in rats. Eur J Pharmacol. 221:77-83.

Morel N, Godfraind T. (1994) The endothelin ETA receptor antagonist, BQ123, normalizes the response of SHR aorta to Ca2+ channel activator. Eur J Pharmacol. 252:R3-R4.

Moreland S, McMullen DM, Delaney CL, Lee VG, Hunt JT. (1992) Venous smooth muscle contains vasoconstrictor ETB-like receptors. Biochem Biophys Res Commun. 184:100-106.

Nakagomi S, Kiryu-seo S, Kiyama H. (2000) Endothelin-converting enzymes and endothelin receptor B messenger RNAs are expressed in different neural cell species and these messenger RNAs are coordinately induced in neurons and astrocytes respectively following nerve injury. Neuroscience. 101:441-449.

Paxinos G, Watson C. (1998) The rat brain in stereotaxic coordinates, 4th ed. San Diego: Academic Press.

Perreault T, Baribeau J. (1995) Characterization of endothelin receptors in newborn piglet lung. Am J Physiol. 268(4 Pt 1):L607-14.

Peters CM, Rogers SD, Pomonis JD, Schmidt JA, Ghilardi JR, Mantyh PW. (2001) Endothelin receptors in the CNS: cell type expression, localization, and involvement in central mechanisms of gliosis. Soc Neurosci Abstr. 27:2046.

Petrov T, Rafols JA. (2002) Acute alterations of endothelin-1 and iNOS expression and control of the brain microcirculation after head trauma. Neurol Res 23:139-143.

Petrov T, Page AB, Owen CR, Rafols JA. (2000) Expression of the inducible nitric oxide synthase in distinct cellular types after traumatic brain injury: an in situ hybridization and immunocytochemical study. Acta Neuropathol (Berl). 100(2):196-204.

Petrov T, Steiner J, Braun B, Rafols JA. (2002a) Sources of endothelin-1 in hippocampus and cortex following traumatic brain injury. Neuroscience. 115(1):275-83.

Petrov T, Krukoff TL, Jhamandas JH. (1992) The hypothalamic paraventricular and lateral parabrachial nuclei receive collaterals from raphe nucleus neurons: a combined double retrograde and immunocytochemical study. J Comp Neurol. 318(1):18-26.

Petrov T, Seitanidou T, Triller A, Korn H. (1991) Differential distribution of GABA- and serotonin-containing afferents on an identified central neuron. Brain Res. 559(1):75-81.

Pollock DM, Schneider MP. (2006) Clarifying endothelin type B receptor function. Hypertension. 48(2):211-2. Epub 2006 Jun 26.

Povlishock JT, Becker DP, Cheng CLY, Waughan GW. (1983) Axonal change in minor head injury. J Neuropathol. And Expl Neurol. 42:225-242.

Rafols J., Kreipke CW, Petrov T. (2007) Alterations in Cerebral Cortex Microvessels and the Microcirculation in a Rat Model of Traumatic Brain Injury: a Correlative EM and Laser Doppler Flowmetry Study. Neurological Research 29:339-347.

Rafols JA. (2007) Editorial: Microvascular and neuronal responses in a model of diffuse brain injury: therapeutic implications. Neurological Research 29:337.

Randall MD, Douglas SA, Hiley CR. (1989) Vascular activities of endothelin-1 and som alanyl substituted analogues in resistance beds of the rat. Br J Pharmacol. 98:685-699.

Roux S, Breu V, Giller T, Neidhart W, Ramuz H, Coassolo P, Clozel JP, Clozel M. (1997) Ro 61-1790, a new hydrosoluble endothelin antagonist: general pharmacology and effects on experimental cerebral vasospasm. J Pharmacol Exp Ther. 283(3):1110-8.

Roux S, Loffler BM, Gray GA, Sprecher U, Clozel M, Clozel JP. (1995) The role of endothelin in experimental cerebral vasospasm. Neurosurgery. 37:78-58.

Saida K, Mitsui Y, Ishida N. (1989) A novel peptide, vasoactive intestinal contractor, of a new (endothelin) peptide family. Molecular cloning, expression, and biological activity. J Biol Chem. 264:14613-14616.

Sakai S, Miyauchi T, Sakurai T, Yamaguchi I, Kobayashi M, Goto K, Sugishita Y. (1996) Pulmonary hypertension caused by congestive heart failure is ameliorated by long-term application of an endothelin receptor antagonist. Increased expression of endothelin-1 messenger ribonucleic acid and endothelin-1-like immunoreactivity in the lung in congestive heart failure in rats. J Am Coll Cardiol. 28(6):1580-8.

Sakurai T, Yangaisawa M, Takuwa Y, Miyazaki H, Kimura S, Goto K, Masaki T. (1991) Cloning of a cDNA encoding a non-isopeptide-selective subtype of the endothelin receptor. Biochem Biophys Res Commun. 178:653-663.

Sakurai T, Yanagisawa M, Masaki T. (1992) Molecular characterization of endothelin receptors. Trends in Pharacol Sci. 13:103-108.

Sakurai-Yamashita Y, Niwa M, Yamashita K, Katoka Y, Himeno A, Shigematsu K, Tsutsumi K, Taniyama K. (1997) Endothelin receptors in kainic acid-induced neural lesions of rat brain. Neuroscience. 81:565-577.

Sato M, Noble LJ. (1998) Involvement of the endothelin receptor subtype A in neuronal pathogenesis after traumatic brain injury. Brain Res. 809(1):39-49.

Sato K, Oka M, Hasunuma K, Ohnishi M, Sato K, Kira S. (1995) Effects of separate and combined ETA and ETB blockade on ET-1-induced constriction in perfused rat lungs. Am J Physiol. 269(5 Pt 1):L668-72.

Shen Y, Kou Z, Kreipke CW, Petrov T, Hu J, Haacke EM. (2007) In vivo measurement of tissue damage, oxygen saturation changes and blood flow changes after experimental traumatic brain injury in rats using Susceptibility Weighted Imaging (SWI). Magn Reson Imaging 25:219-227.

Shigeno T, Clozel M, Sakai S, Saito A, Goto K. (1995) The effect of Bosentan, a new potent endothelin receptor antagonist, on the pathogenesis of cerebral vasospasm. Neurosurgery. 37:87-90.

Shin T, Kang B, Tanuma M. Matsumoto Y, Wie M, Ahn M, Kang J. (2001) Intrathecal administration of endothelin-1 receptor antagonist ameliorates autoimmune encephalomyelitis in Lewis rats. Neuroreport. 12:1465-1468.

Teerlink JR, Breu V, Sprecher U, Clozel M, Clozel JP. (1994) Potent vasoconstriction mediated by endothelin ETB receptors in canine coronary arteries. Circ Res. 74:105-114.

Therkelsen K, Jensen KA, Freundlich M, Thorshauge H, BünemannL, Bøgeskov Nielsen L. (1994) Endothelin-1 and cerebral blood flow: influence of hypoxia, hypercapnia and indomethacin on circulating endothelin levels in healthy volunteers. Scand J Clin Lab Invest. 54(6):441-51.

Touzani O, Galabraith S, Siegl P, McCulloch J. (1997) Endothelin-B receptors in cerebral resistance arterioles and their functional significance after focal cerebral ischemia in cats. J Cereb Blood Flow Metab. 17:1157-1165.

Uhlmann D. (2006) Clazosentan (Actelion). Curr Opin Investig Drugs. 7(3):272-81.

Vierhapper H, Wagner O, Nowotny P, Walhäusl W. (1990) Effect of endothelin-1 in man. Circulation. 81(4):1415-8.

Vuurmans JL, Boer P, Koomans HA. (2004) Effects of endothelin-1 and endothelin-1-receptor blockade on renal function in humans. Nephrol Dial Transplant. (11):2742-6.

Warner TD, Allcock GH, Mickley EJ, Corder R, Vane JR. 1993) Comparative studies with the endothelin receptor antagonists BQ-123 and PD 142893 indicate at least three endothelin receptors. J Cardiovasc Pharmacol 22 Suppl. 8:S117-S120.

Warner TD, Allcock GH, Vane JR. (1994a) Reversal of established responses to endothelin-1 in vivo and in vitro by the endothelin receptor antagonists, Bq-123 and PD 145065. Br J Pharmacol. 112:207-213.

Warner TD, Battistini B, Doherty AM, Corder R. (1994b) Endothelin receptor antagonists: actions and rationale for their development. Biochem Pharmacol. 48:625-635.

Wei H, Liang G, Yang H. (2007) Isoflurane preconditioning inhibited isoflurane-induced neurotoxicity. Neurosci Lett. 425(1):59-62.

Williams DL Jr, Jones KL, Pettibone DJ, Lis EV, Clineschmidt BV. (1991) Sarafotoxin S6c: an agonist which distinguishes between endothelin receptor subtypes. Biochem Biophys Res Commun. 175:556-561.

Winder SJ, Walsh MP. (1990) Smooth muscle calponin. Inhibition of actomyosin MgATPase and regulation by phosphorylation. J Biol Chem. 265(17):10148-55.

Worth NF, Rolfe BE, Song J, Campbell GR. (2001) Vascular smooth muscle cell phenotypic modulation in culture is associated with reorganisation of contractile and cytoskeletal proteins. Cell Motil Cytoskeleton.49(3):130-45.

Yanagisawa M, Kurihara H, Kimura AS, Tomobe Y, Kobayashi M, Mitsui Y, Yazaki Y, Goto K, Masaki T. (1988) A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature. 332:411-415.

Yanagisawa M, Masaki T. (1989) Molecular biology and biochemistry of the endothelins. Trends Pharmacol Sci. 10:374-378.

Yip AW, Krukoff TL. (2002) Endothelin-A receptors and NO mediate decrease in arterial pressure during recovery from resistant. Am J Physiol Regul Integr Comp Physiol. 282:R881-889.

Zimmermann M, Seifert V. (1998) Endothelin and subarachnoid hemorrhage: an overview. Neurosurgery. 43:863-875.

Zhao P, Peng L, Li L, Xu X, Zuo Z. (2007) Isoflurane preconditioning improves long-term neurologic outcome after hypoxic-ischemic brain injury in neonatal rats. Anesthesiology. 107(6):963-70.

PHS 398 Checklist

OMB Number: 0925-0001
Expiration Date: 9/30/2007

1. Application Type:

From SF 424 (R&R) Cover Page. The responses provided on the R&R cover page are repeated here for your reference, as you answer the questions that are specific to the PHS398.

* Type of Application:

☐ New ☒ Resubmission ☐ Renewal ☐ Continuation ☐ Revision

Federal Identifier: NS039860

2. Change of Investigator / Change of Institution Questions

☐ Change of principal investigator / program director

Name of former principal investigator / program director:

Prefix:
* First Name:
Middle Name:
* Last Name:
Suffix:

☐ Change of Grantee Institution

* Name of former institution:

3. Inventions and Patents (For renewal applications only)

* Inventions and Patents: Yes ☐ No ☒

If the answer is "Yes" then please answer the following:

* Previously Reported: Yes ☐ No ☐

OMB Number. 0925-0001
Expiration Date: 9/30/2007

4. * Program Income

Is program income anticipated during the periods for which the grant support is requested?

☐ Yes ☒ No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period	*Anticipated Amount ($)	*Source(s)

5. Assurances/Certifications (see instructions)

In agreeing to the assurances/certification section 18 on the SF424 (R&R) form, the authorized organizational representative agrees to comply with the policies, assurances and/or certifications listed in the agency's application guide, when applicable. Descriptions of individual assurances/certifications are provided at: http://grants.nih.gov/grants/funding/424

If unable to certify compliance , where applicable, provide an explanation and attach below.

Explanation: Add Attachment Delete Attachment View Attachment